US011291708B2

(12) **United States Patent**
Arce

(10) **Patent No.: US 11,291,708 B2**
(45) **Date of Patent: *Apr. 5, 2022**

(54) **HUMAN-DERIVED RECOMBINANT FSH FOR CONTROLLED OVARIAN STIMULATION**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **16/851,260**

(22) Filed: **Apr. 17, 2020**

(65) **Prior Publication Data**

US 2020/0345814 A1 Nov. 5, 2020

Related U.S. Application Data

(63) Continuation of application No. 15/637,962, filed on Jun. 29, 2017, now Pat. No. 10,624,953, which is a continuation of application No. 14/237,697, filed as application No. PCT/EP2012/065507 on Aug. 8, 2012, now Pat. No. 9,694,052.

(30) **Foreign Application Priority Data**

Aug. 8, 2011 (EP) 1176803

(51) **Int. Cl.**
A61K 38/24 (2006.01)

(52) **U.S. Cl.**
CPC ***A61K 38/24*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Preparations including FSH, for example recombinant FSH, for use in the treatment of infertility.

8 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

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Fig 1: FSH expression vector

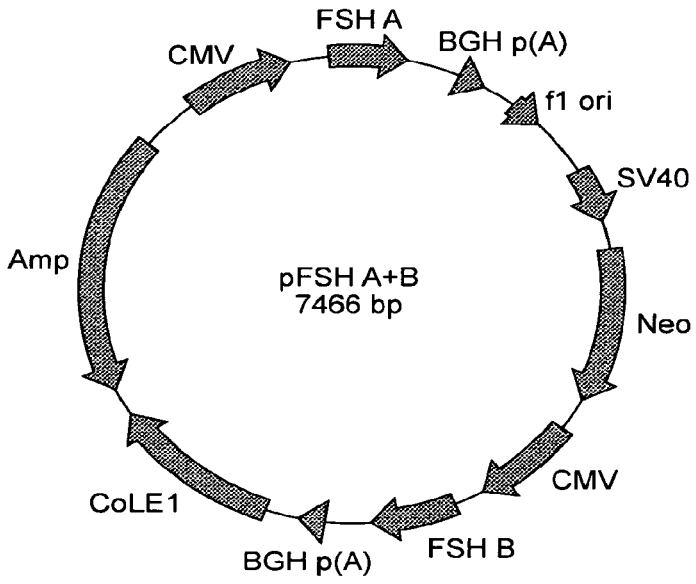

FIG. 1

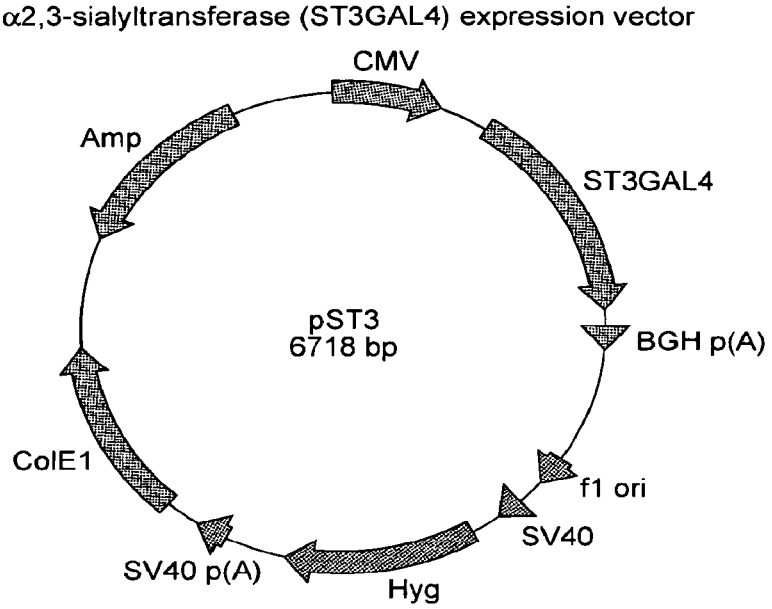
α2,3-sialyltransferase (ST3GAL4) expression vector
CMV
ST3GAL4
BGH p(A)
f1 ori
SV40
Hyg
SV40 p(A)
ColE1
Amp
pST3
6718 bp

FIG. 2

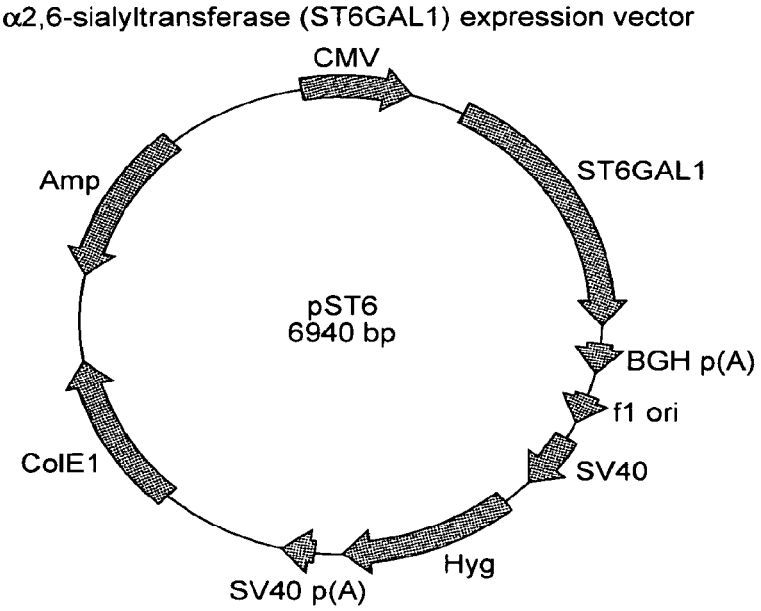
α2,6-sialyltransferase (ST6GAL1) expression vector
CMV
ST6GAL1
BGH p(A)
f1 ori
SV40
Hyg
SV40 p(A)
ColE1
Amp
pST6
6940 bp

FIG. 3

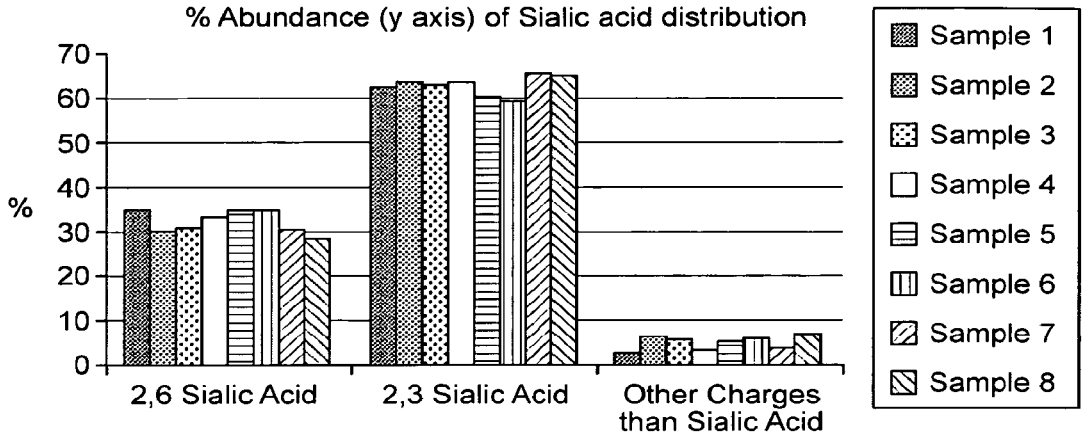
% Abundance (y axis) of Sialic acid distribution
%
70
60
50
40
30
20
10
0
2,6 Sialic Acid
2,3 Sialic Acid
Other Charges than Sialic Acid
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8

FIG. 4

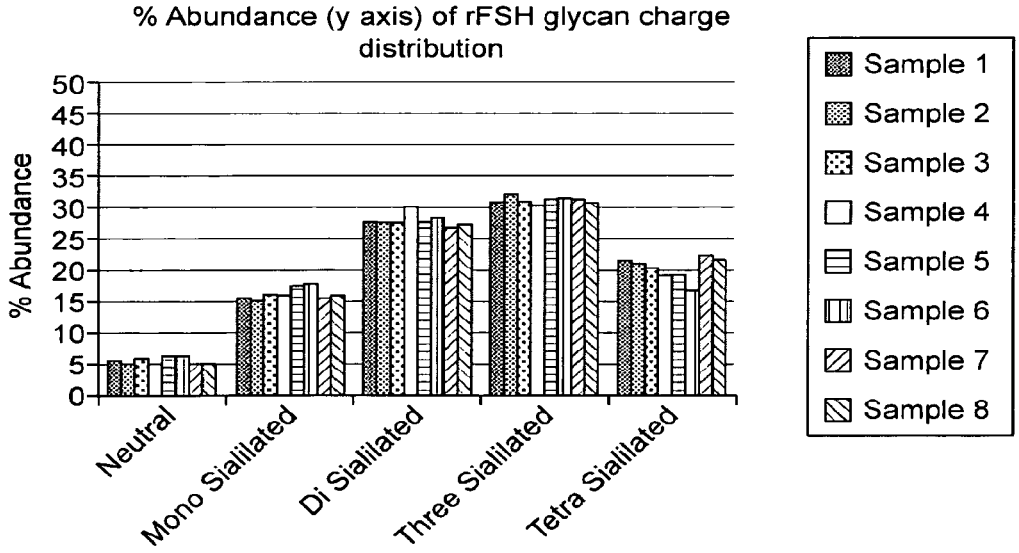
% Abundance (y axis) of rFSH glycan charge distribution
% Abundance
50
45
40
35
30
25
20
15
10
5
0
Neutral
Mono Sialilated
Di Sialilated
Three Sialilated
Tetra Sialilated
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8

FIG. 5

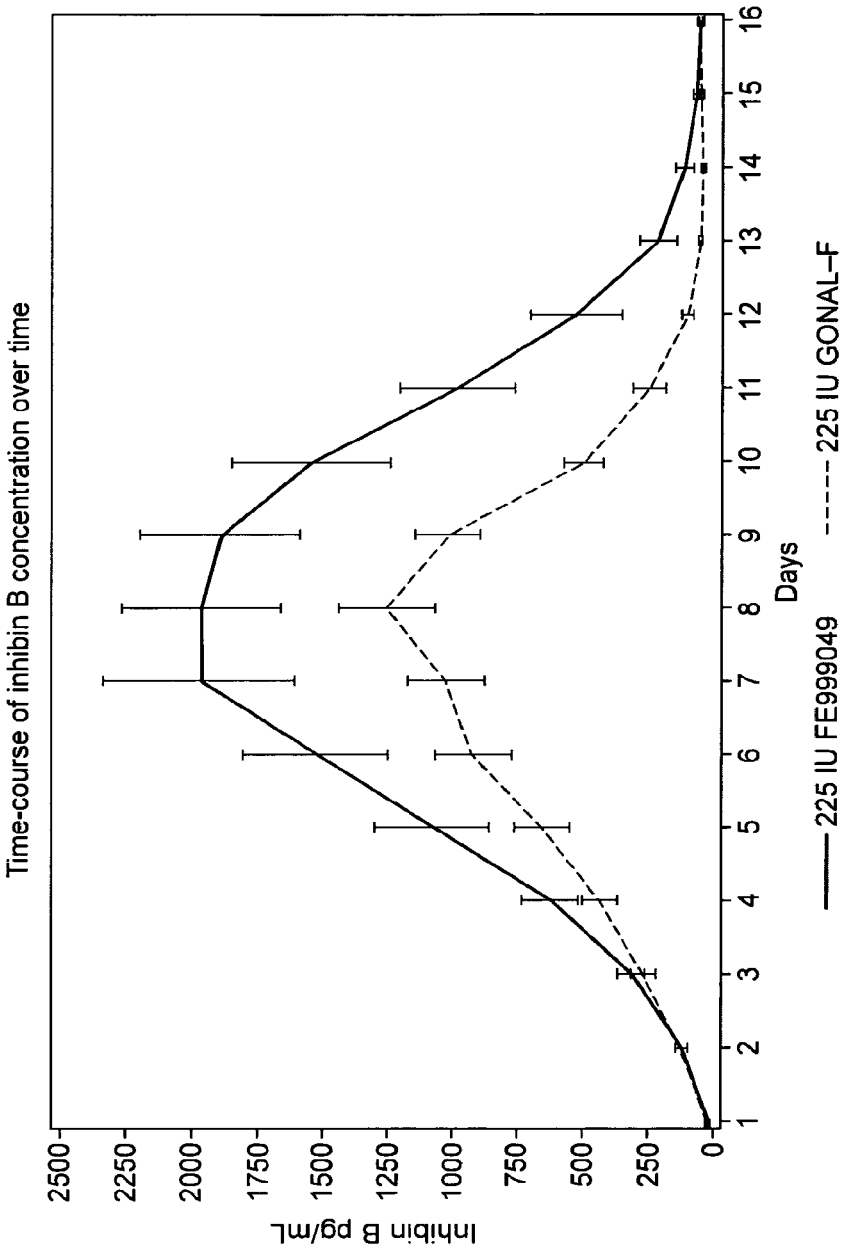
Time-course of inhibin B concentration over time
Inhibin B pg/mL
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Days
225 IU FE999049
225 IU GONAL-F

FIG. 6

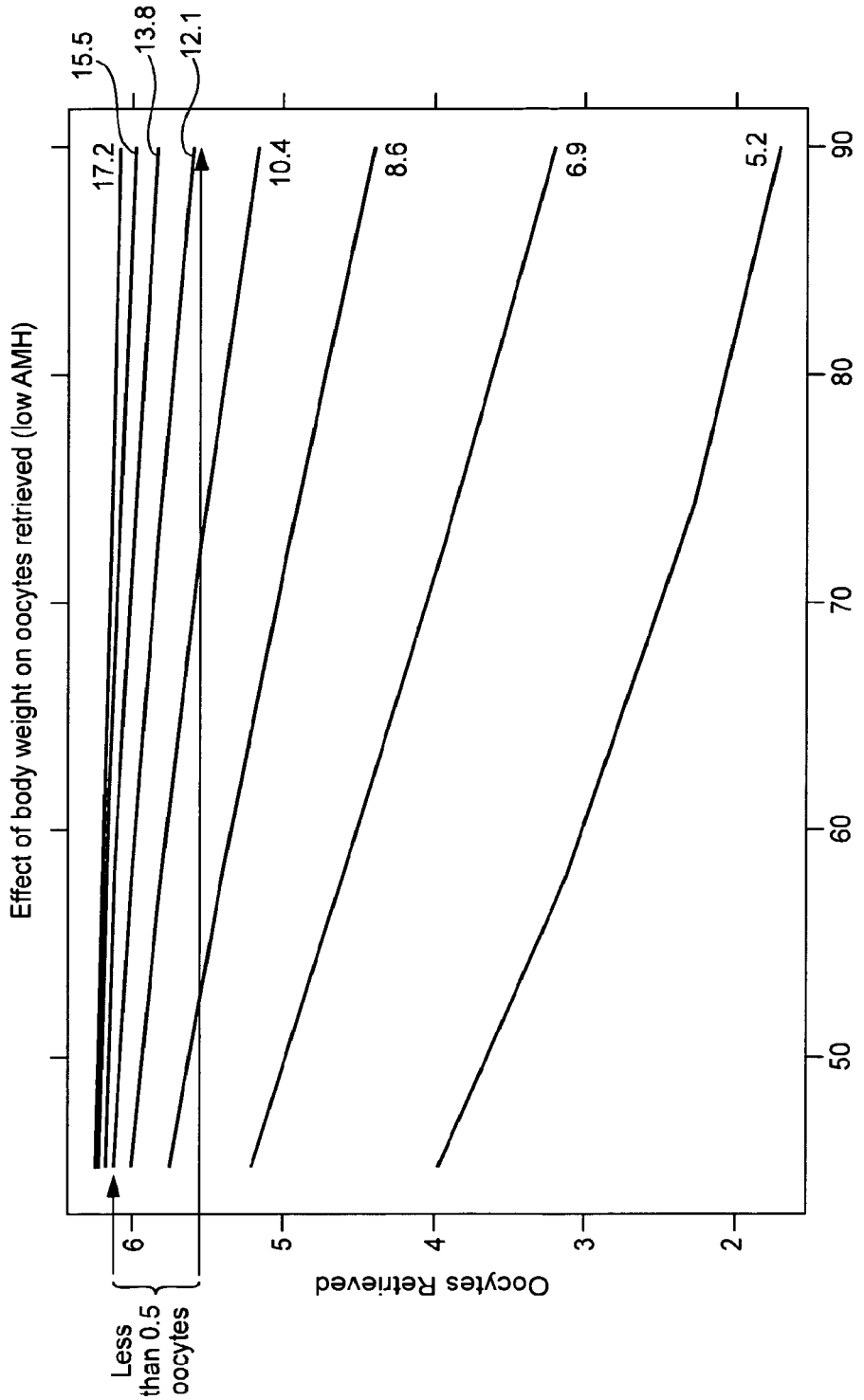
Effect of body weight on oocytes retrieved (low AMH)
Oocytes Retrieved
Weight (kg)
15.5
13.8
12.1
17.2
10.4
8.6
6.9
5.2
Less than 0.5 oocytes
2
3
4
5
6
50
60
70
80
90

FIG. 7

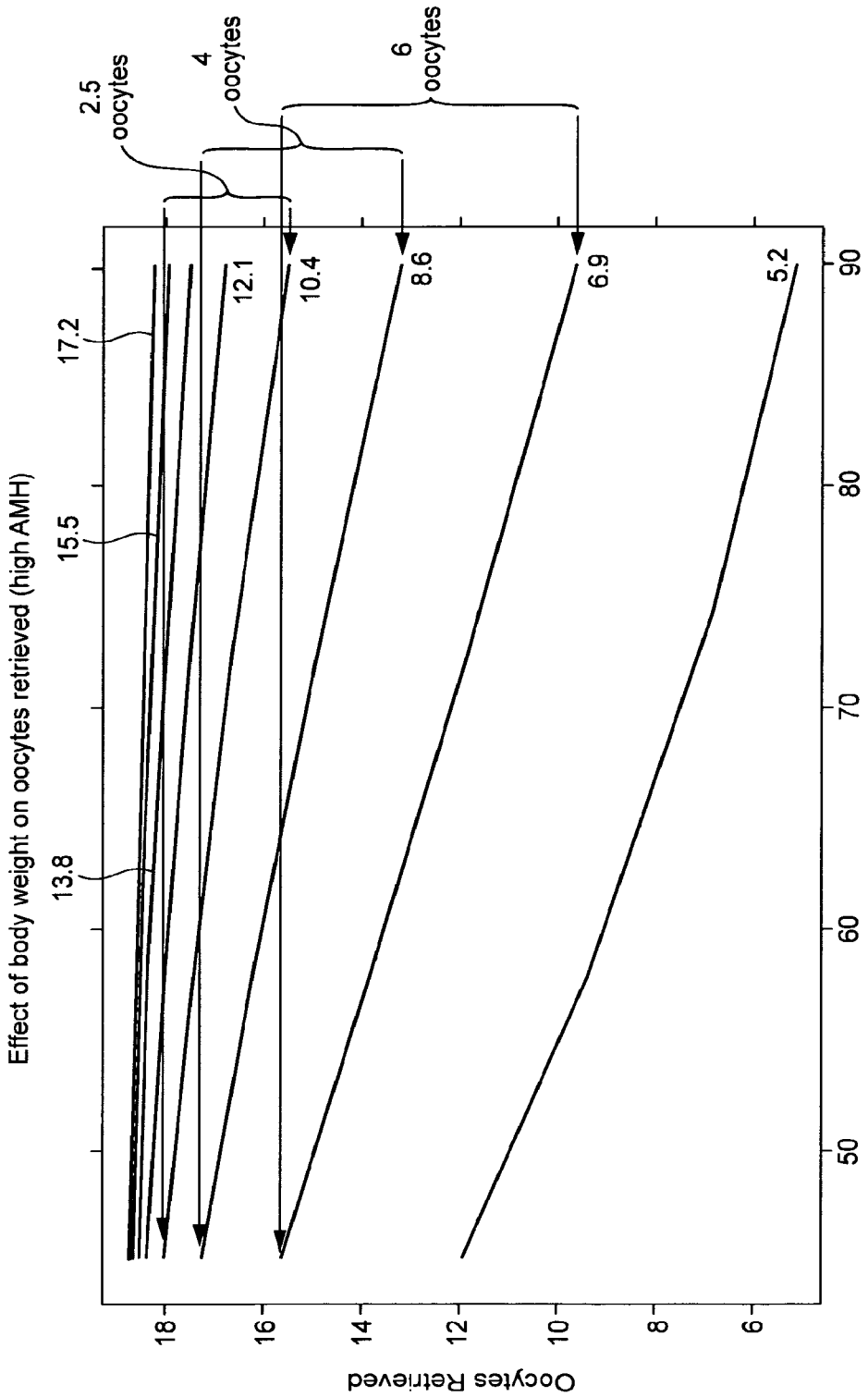
Effect of body weight on oocytes retrieved (high AMH)
2.5 oocytes
4 oocytes
6 oocytes
17.2
15.5
13.8
12.1
10.4
8.6
6.9
5.2
Oocytes Retrieved
Weight (kg)
18
16
14
12
10
8
6
50
60
70
80
90

FIG. 8

HUMAN-DERIVED RECOMBINANT FSH FOR CONTROLLED OVARIAN STIMULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application a continuation of U.S. application Ser. No. 15/637,962, filed Jun. 29, 2017, which is a continuation of U.S. application Ser. No. 14/237,697, filed Jun. 30, 2014, which is the U.S. National Stage of International Application No. PCT/EP2012/065507, filed Aug. 8, 2012, and claims priority to European Patent Application No. 11176803.2, filed Aug. 8, 2011.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.

The present invention relates to compositions and pharmaceutical products for the treatment of infertility.

Assisted reproductive technology (ART) techniques such as in vitro fertilisation (IVF) are well known. These ART techniques generally require a step of controlled ovarian stimulation (COS), in which a cohort of follicles is stimulated to full maturity. Standard COS regimens include administration of gonadotrophins, such as follicle stimulating hormone (FSH) alone or in combination with luteinising hormone (LH) activity to stimulate follicular development, normally with administration of a GnRH analogue prior to and/or during stimulation to prevent premature LH surge. The pharmaceutical compositions generally used for COS include recombinant follicle stimulating hormone (rFSH), urinary derived FSH, recombinant FSH+LH preparations, urinary derived menotrophin [human menopausal gonadotrophin (hMG)] and highly purified human menopausal gonadotrophin (HP-hMG). IVF can be associated with a risk of ovarian hyperstimulation syndrome (OHSS), which can be life threatening in severe cases.

The ability to predict the response potential of women to controlled ovarian stimulation (COS) may allow the development of individualised COS protocols. This could, for example, reduce the risk of OHSS in women predicted to have an excessive response to stimulation, and/or improve pregnancy outcomes in women classed as poor responders. The serum concentration of anti-Müllerian hormone (AMH) is now established as a reliable marker of ovarian reserve. Decreasing levels of AMH are correlated with reduced ovarian response to gonadotrophins during COS. Further, high levels of AMH are a good predictor of excessive ovarian response, and an indicator of risk of OHSS.

In a preliminary study of women under 35 years old undergoing ART, the CONSORT dosing algorithm (incorporating basal FSH, BMI, age and AFC) was used to predict the optimal FSH starting dose for COS in women at risk of developing OHSS (Olivennes et. al., 2009). Individualising the dose did lead to adequate oocyte yield and good pregnancy rate. However, there were high rates of cancellations in the low dose group (75 IU FSH) due to inadequate response, and OHSS did occur in a significant proportion of the patients.

There is therefore a need for a composition for use in individualised COS protocols which provides adequate response to stimulation, and/or decreased risk of OHSS.

As indicated above, standard COS protocols may include administration of FSH. FSH is naturally secreted by the anterior pituitary gland and functions to support follicular development and ovulation. FSH comprises a 92 amino acid alpha sub-unit, also common to the other glycoprotein hormones LH and CG, and a 111 amino acid beta sub-unit unique to FSH that confers the biological specificity of the hormone (Pierce and Parsons, 1981). Each sub-unit is post translationally modified by the addition of complex carbohydrate residues. Both subunits carry 2 sites for N-linked glycan attachment, the alpha sub-unit at amino acids 52 and 78 and the beta sub-unit at amino acid residues 7 and 24 (Rathnam and Saxena, 1975, Saxena and Rathnam, 1976). FSH is thus glycosylated to about 30% by mass (Dias and Van Roey. 2001. Fox et al. 2001).

FSH purified from post-menopausal human urine has been used for many years in infertility treatment; both to promote ovulation in natural reproduction and to provide oocytes for assisted reproduction technologies. The currently approved recombinant FSH (rFSH) products for ovarian stimulation, such as follitropin alfa (GONAL-F, Merck Serono/EMD Serono) and follitropin beta (PUREGON/FOLLISTIM, MSD/Schering-Plough), are derived from a Chinese Hamster Ovary (CHO) cell line. Currently, no rFSH products from a human cell line are commercially available.

There is considerable heterogeneity associated with FSH preparations which relates to differences in the amounts of various isoforms present. Individual FSH isoforms exhibit identical amino acid sequences but differ in the extent to which they are post-translationally modified; particular isoforms are characterised by heterogeneity of the carbohydrate branch structures and differing amounts of sialic acid (a terminal sugar) incorporation, both of which appear to influence the specific isoform bioactivity.

Glycosylation of natural FSH is highly complex. The glycans in naturally derived pituitary FSH can contain a wide range of structures that can include combinations of mono-, bi-, tri- and tetra-antennary glycans (Pierce and Parsons, 1981. Ryan et al., 1987. Baenziger and Green, 1988). The glycans can carry further modifications: core fucosylation, bisecting glucosamine, chains extended with acetyl lactosamine, partial or complete sialylation, sialylation with α2,3 and α2,6 linkages, and sulphated galactosamine substituted for galactose (Dalpathado et al., 2006). Furthermore, there are differences between the distributions of glycan structures at the individual glycosylation sites. A comparable level of glycan complexity has been found in FSH derived from the serum of individuals and from the urine of post-menopausal women (Wide et al., 2007).

The glycosylation of recombinant FSH products reflects the range of glycosyl-transferases present in the host cell line. The commercially available rFSH products are derived from engineered Chinese hamster ovary cells (CHO cells). The range of glycan modifications in CHO cell derived rFSH are more limited than those found on the natural products. Examples of the reduced glycan heterogeneity found in CHO cell derived rFSH include a lack of bisecting glucosamine and a reduced content of core fucosylation and acetyl lactosamine extensions (Hard et al., 1990). In addition, CHO cells are only able to add sialic acid using the α2,3 linkage (Kagawa et al, 1988, Takeuchi et al, 1988, Svensson et al., 1990); CHO cell derived rFSH only includes α2,3-linked sialic acid and does not include α2,6-linked sialic acid.

Thus CHO cell derived FSH is different from naturally produced FSH (e.g. human Pituitary/serum/urinary FSH) which contains glycans with a mixture of α2,3 and α2,6-linked sialic acid, with a predominance of the former.

Further, it has also been demonstrated that the commercially available recombinant FSH preparation differs in the amounts of FSH with an isoelectric point (pI) of below 4 (considered the acidic isoforms) when compared to pituitary, serum or post-menopausal urine FSH (Ulloa-Aguirre et al. 1995). The amount of acidic isoforms in the urinary preparations was much higher as compared to the CHO cell derived recombinant products, Gonal-f (Merck Serono) and Puregon (Schering Plough) (Andersen et al. 2004). This must reflect a lower molar content of sialic acid in the recombinant FSH since the content of negatively-charged glycan modified with sulphate is low in recombinant FSH. The lower sialic acid content, compared to natural FSH, is a feature of both commercially available recombinant FSH products and may reflect a limitation in the manufacturing process.

The circulatory life-time of FSH has been documented for materials from a variety of sources. Some of these materials have been fractionated on the basis of overall molecular charge, as characterised by their pI, in which more acid equates to a higher negative charge. As previously stated the major contributor to overall molecular charge is the total sialic content of each FSH molecule. For instance, rFSH (Organon) has a sialic acid content of around 8 mol/mol, whereas urine-derived FSH has a higher sialic acid content (de Leeuw et al. 1996). The corresponding plasma clearance rates in the rat are 0.34 and 0.14 ml/min (Ulloa-Aguirre et al. 2003). In another example where a sample of recombinant FSH was split into high and low pI fractions, the in vivo potency of the high pI (lower sialic acid content) fraction was decreased and it had a shorter plasma half-life (D'Antonio et al. 1999). It has also been reported that the more basic FSH circulating during the later stages of the ovulation cycle is due to the down-regulation of α2,3 sialyl-transferase in the anterior pituitary which is caused by increasing levels of estradiol (Damian-Matsumara et al. 1999. Ulloa-Aguirre et al. 2001). Results for the α2,6 sialyl-transferase have not been reported.

Thus, as set out above, recombinant proteins expressed using the CHO system will differ from their natural counterparts in their type of terminal sialic acid linkages. This is an important consideration in the production of biologicals for pharmaceutical use since the carbohydrate moieties may contribute to the pharmacological attributes of the molecule. The present applicants have developed a human derived recombinant FSH which is the subject of International Patent Application No. PCT/GB2009/000978, published as WO2009/127826A. Recombinant FSH with a mixture of both α2,3 and α2,6-linked sialic acid was made by engineering a human cell line to express both rFSH and α2,3 sialyltransferase. The expressed product is highly acidic and carries a mix of both α2,3- and α2,6-linked sialic acids; the latter provided by the endogenous sialyl transferase activity. It was found that the type of sialic acid linkage, α2,3- or α2,6-, can have a dramatic influence on biological clearance of FSH. Recombinant FSH with a mixture of both α2,3 and α2,6-linked sialic acid has two advantages over rFSH expressed in conventional CHO cells: first the material is more highly sialylated due to the combined activities of the two sialyltransferases; and secondly the material more closely resembles the natural FSH. This is likely to be more biologically appropriate compared to CHO cell derived recombinant products that have produce only α2,3 linked sialic acid (Kagawa et al, 1988, Takeuchi et al, 1988, Svensson et al., 1990) and have decreased sialic acid content (Ulloa-Aguirre et al. 1995, Andersen et al. 2004).

The rFSH product disclosed in International Patent Application No. PCT/GB2009/000978 contains branched glycan moieties. FSH comprises glycans (attached to the FSH glycoproteins) and these glycans may contain a wide variety of structures. As is well known in the art, branching (of a glycan) can occur with the result that the glycan may have 1, 2, 3, 4 or more terminal sugar residues or "antennae"; glycans with 1, 2, 3 or 4 terminal sugar residues or "antennae" are referred to respectively as mono-antennary, di-antennary, tri-antennary or tetra-antennary structures. Glycans may have sialylation presence on mono-antennary and/or di-antennary and/or tri-antennary and/or tetra-antennary structures. An example rFSH disclosed in International Patent Application No. PCT/GB2009/000978 included mono-sialylated, di-sialylated, tri-sialylated and tetra-sialylated glycan structures with relative amounts as follows: 9-15% mono-sialylated; 27-30% di-sialylated; 30-36% tri-sialylated and 25-29% tetra-sialylated. As is well known, a mono-sialylated glycan structure carries one sialic acid residue; a di-sialylated glycan structure carries two sialic acid residues; a tri-sialylated glycan structure carries three sialic acid residues; and a tetra-sialylated glycan structure carries four sialic acid residues. Herein, terminology such as "X % mono-sialylated", "X % di-sialylated", "X % tri-sialylated" or "X % tetra-sialylated" refers to the number of glycan structures on FSH which are mono-, di, tri or tetra sialylated (respectively), expressed as a percentage (X %) of the total number of glycan structures on the FSH which are sialylated in any way (carry sialic acid). Thus, the phrase "30-36% tri-sialylated glycan structures" means that, of the total number of glycan structures on the FSH which carry sialic acid residues (that is, are sialylated), 30 to 36% of these glycan structures are tri sialylated (carry three sialic acid residues). The applicants have surprisingly found that FSH having a specific amount of tetra-sialylated glycan structures (which is different to that of the example rFSH product disclosed in PCT/GB2009/000978 mentioned above) is markedly more potent then recombinant FSH products which are currently on the market. The amino acid sequence of the applicant's products is the native sequence and is identical to natural human FSH and existing CHO-derived rFSH products. However, the present applicants have found that human derived recombinant FSH products (i.e. recombinant FSH produced or expressed in a human cell line e.g. made by engineering a human cell line) which have a mixture of both α2,3 and α2,6-linked sialic acid and/or a specific amount of tetra-sialylated glycan structures may be particularly effective when utilised in (e.g. individualised) COS protocols.

According to the present invention in a first aspect there is provided a product (e.g. a pharmaceutical composition) comprising follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient (e.g. a patient having serum AMH level of 0.05 pmol/L or above, for example 0.5 pmol/L or above), wherein the product comprises a dose of, or a dose equivalent to, 1-24 μg, for example 2-24 μg, for example 2 to 15 μg, human derived recombinant FSH. Preferably the product comprises a dose of, or a dose equivalent to, 4.5 to 12.5 μg, for example 5 to 12.5 μg, for example 6 to 12.5 μg, for example 6.3 to 10.5 μg, human derived recombinant FSH.

According to the invention there is provided a product (e.g. a pharmaceutical composition) comprising follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient having serum AMH level of <15 pmol/L (e.g. 0.05 pmol/L to 14.9 pmol/L), wherein the product comprises a (e.g. daily) dose of, or dose equivalent

to, 9 to 14 μg, for example 11 to 13 μg, for example 12 μg human derived recombinant FSH. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The dose provides an effective response while minimising risk of OHSS. Preferably the treatment of infertility comprising a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of <15 pmol/L (e.g. 0.05 pmol/L to 14.9 pmol/L).

According to the invention in a further aspect there is provided a product (e.g. a pharmaceutical composition) comprising follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient having serum AMH level of ≥15 pmol/L, wherein the product comprises a (e.g. daily) dose of, or dose equivalent to, 5 to 12.5 μg, for example 6 to 10.5 μg human derived recombinant FSH. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The dose provides an effective response while minimising risk of OHSS. Preferably the treatment of infertility comprising a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of ≥15 pmol/L. In one embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of 15 to 24.9 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 5 to 12 μg, for example 7 to 12 μg, for example 8.7 to 10 μg, human derived recombinant FSH (preferably 9 to 10 μg human derived recombinant FSH) In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of 15 to 24.9 pmol/L. In another embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of 25 to 34.9 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 5 to 12 μg, for example 6 to 9 μg, for example 7 to 8 μghuman derived recombinant FSH (preferably 7.3 to 8 μg human derived recombinant FSH). In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of 25 to 34.9 pmol/L. In another embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of ≥35 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 5 to 11 μg, for example 6.3 to 7 μg, human derived recombinant FSH (preferably 6 to 7 μg human derived recombinant FSH). In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of ≥35 pmol/L.

The doses above may be for treatment of infertility in the patient's (subject's) first stimulation protocol. It will be appreciated that for further stimulation cycles, the doses may be adjusted according to actual ovarian response in the first cycle.

The applicants have found that it is generally necessary to retrieve in the region of nine oocytes in order to enable selection of two high quality oocytes for transfer.

The applicants have found that for subjects having low AMH (AMH <15 pmol/L per litre) a reasonably high dose of recombinant FSH is required (for example 12 μg) to achieve this. At this dose, 8 to 14 oocytes will be retrieved from 60% of subjects with low AMH. This is an unexpected and significant improvement over treatment of subjects with low AMH treated with 150 IU Gonal-f, where 8 to 14 oocytes are retrieved from only 33% of subjects. The applicants have found that there is no need to adjust this dose according to the bodyweight of the patient.

However, 60% of the population (and 80% of women under 30 treated for infertility) have high AMH (that is, AMH of 15 pmol/L). For these subjects it is generally fairly straightforward to retrieve a mean of 9 to 11 oocytes; the problem with stimulation protocols is the risk of OHSS. The applicants have found that in patients dosed at low doses of human recombinant FSH that there is a relationship between oocytes retrieved and body weight of the subject. This means that there may be a risk associated with treatment with a fixed dose of FSH (which is usual in the art). The present applicants have established a relationship between dose of FSH and AMH level and weight of the subject which provides an improved safety profile (reduced risk of OHSS) with acceptable or improved oocyte retrieval compared to the known treatment protocols (see example 10).

According to the invention in a further aspect there is provided a product (e.g. a pharmaceutical composition) comprising follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient having serum AMH level of ≥15 pmol/L, wherein the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 0.09 to 0.19 μg (for example 0.09 to 0.17 μg) human derived recombinant FSH per kg bodyweight of the patient. Preferably the treatment of infertility comprises a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of 215 pmol/L. In one embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of 15 to 24.9 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 0.14 to 0.19 μg human derived recombinant FSH (preferably 0.15 to 0.16 μg human derived recombinant FSH) per kg bodyweight of the patient. In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of 15 to 24.9 pmol/L. In another embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of 25 to 34.9 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 0.11 to 0.14 μg human derived recombinant FSH (preferably 0.12 to 0.13 μg human derived recombinant FSH) per kg bodyweight of the patient. In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of 25 to 34.9 pmol/L. In a still further embodiment, the product is for use in the treatment of infertility in a patient having serum AMH level of ≥35 pmol/L, and the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 0.10 to 0.11 μg human derived recombinant FSH per kg bodyweight of the patient. In this embodiment, the treatment of infertility may comprise a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of ≥35 pmol/L. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The doses provide an effective response while minimising risk of OHSS.

The doses above may be for treatment of infertility in the patient's (subject's) first stimulation protocol. It will be

appreciated that for further stimulation cycles, the doses may be adjusted according to actual ovarian response in the first cycle.

According to the invention in a still further aspect there is provided a product (e.g. a pharmaceutical composition) comprising follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient having serum AMH level of <15 pmol/L, wherein the product is for administration at a (e.g. daily) dose of, or dose equivalent to, 0.15 to 0.21 μg, (for example 0.19 to 0.21 μg) human derived recombinant FSH per kg bodyweight of the patient. Preferably the treatment of infertility comprises a step of determining (e.g. measuring) the serum AMH level of the patient, and administering the dose to a patient having serum AMH level of <15 pmol/L.

However, it is not required that patients having serum AMH level of <15 pmol/L are dosed by body weight. It will be appreciated that these doses may be readily converted to treat patients with dosing according to their BMI, using conversions well known in the art. The product (e.g. pharmaceutical compostion) may be for use in the treatment of infertility in a patient having serum AMH of 5.0-14.9 pmol/L, wherein the product comprises a dose of, or dose equivalent to, 6 to 18 μg, for example 8 to 11 μg, for example 8.5 to 10.2 μg human derived recombinant FSH. The product may be for use in the treatment of infertility in a patient having serum AMH 15.0-29.9 pmol/L, wherein the product comprises a dose of, or a dose equivalent to, 4.8 to 15 μg, for example 6 to 9 μg, for example 6.8 to 8.5 μg human derived recombinant FSH. The product may be for use in the treatment of infertility in a patient having serum AMH 30-44.9 pmol/L, wherein the product comprises a dose of, or a dose equivalent to, 3.6 to 12 μg, for example 4 to 7 μg, for example 5.1 to 6.8 μg human derived recombinant FSH. The product may be for use in the treatment of infertility in a patient having serum AMH 45 pmol/L or greater, wherein the product comprises a dose of, or a dose equivalent to, 2 to 9 μg, for example 2.4 to 9 μg (for example 3.4 to 5.1 μg) or 2 to 5 μg human derived recombinant FSH. The product may comprise follicle stimulating hormone (FSH) for use in the treatment of infertility in a patient having serum AMH of 5 pmol/L or less, wherein the product comprises a dose of, or a dose equivalent to 7.2 to 24 μg, for example 10 to 15 μg for example 10.2 to 13.6 μg, human derived recombinant FSH. The product may be for use in the treatment of infertility in a patient wherein the product comprises a dose of, or dose equivalent to, 4.8 to 18 μg, for example 6 to 11 μg, for example 6.8 to 10.2 μg human derived recombinant FSH. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH.

Preferably the rFSH (e.g. human cell line derived recombinant FSH) includes α2,3- and α2,6-sialylation. The FSH (rFSH) for use according to the invention may have 1% to 99% of the total sialylation being α2,3-sialylation. The FSH (rFSH) according to the invention may have 1% to 99% of the total sialylation being α2,6-sialylation. Preferably, 50 to 70%, for example 60 to 69%, for example about 65%, of the total sialylation is α2,3-sialylation. Preferably 25 to 50%, for example 30 to 50%, for example 31 to 38%, for example about 35%, of the total sialylation is α2,6-sialylation.

Preferably the rFSH (e.g. human cell line derived recombinant FSH) includes mono-, di-, tri- and tetra-sialylated glycan structures, wherein 15-24%, for example 17-23% of the sialylated glycan structures are tetrasialylated glycan structures (e.g. as shown by WAX analysis of charged glycans, as set out in the Examples below). The FSH comprises glycans (attached to the FSH glycoproteins). It is well known that glycans in FSH may contain a wide variety of structures. These may include combinations of mono, bi, tri and tetra-antennary glycans. Herein, terminology such as "X % of the sialylated glycan structures are tetrasialylated glycan structures" refers to the number of glycan structures on the FSH which are tetra sialylated, i.e. carry four sialic acid residues, expressed as a percentage (X %) of the total number of glycan structures on the FSH which are sialylated in any way (carry sialic acid). Thus, the phrase "15-24% of the sialylated glycan structures are tetrasialylated glycan structures" means that, of the total number of glycan structures on FSH which carry sialic acid residues (that is, are sialylated), 15 to 24% of these glycan structures are tetra sialylated (carry four sialic acid residues).

The rFSH may be present as a single isoform or as a mixture of isoforms.

The applicants have devised "individualised" COS protocols wherein specific doses of recombinant FSH having specific characteristics are used to treat patients based on their specific AMH levels, thereby increasing the likelihood of adequate response to stimulation (e.g. in patients having a low response potential), and/or decreased risk of OHSS (e.g. in patients classed as high or excessive responders).

The serum level of AMH may be determined (e.g. measured) by any method known in the art. Preferably the serum AMH level is measured using the AMH Gen-II enzyme linked immunosorbent assay, a kit (Beckman Coulter, Inc., Webster, Tex.). This assay can detect can detect AMH concentrations greater than 0.57 pmol/L with a minimum limit of quantitation of 1.1 pmol/L. Other assays may be used.

Herein, serum AMH values are generally recited in terms of pmol/L. This may be converted to ng/mL using the conversion equation 1 ng/ml AMH=7.1. pmol/L AMH.

Herein the terms "patient" and "subject" are used interchangeably.

The product (e.g. pharmaceutical composition) preferably comprises a daily dose of, or a daily dose equivalent to, the amounts of human derived rFSH defined above, herein, and in the claims. The (daily) dose may be an initial dose (i.e. it may be reduced, increased, or maintained during the treatment).

The product (e.g. pharmaceutical composition) may be for (daily) administration of FSH starting on day one of treatment and continuing for seven to thirteen days, for example nine to thirteen days, for example 10 to 13 days, for example 10 to 11 days. The product (e.g. pharmaceutical composition) may be for administration 12 to 16, e.g. 13 to 15, e.g. 14 days after administration of (e.g. after initiation of administration of, e.g. after initiation of daily administration of) a GnRH agonist (e.g. Synarel, Lupron, Decapeptyl). The product (e.g. pharmaceutical composition) may be for administration with a GnRH agonist. The product (e.g. pharmaceutical composition) may be for administration prior to administration of a GnRH antagonist (e.g. ganirelix, cetrorelix), for example for administration five or six days prior to administration of a GnRH antagonist. The product (e.g. pharmaceutical composition) may be for administration with a GnRH antagonist. Preferably the product (e.g. pharmaceutical composition) is for administration prior to administration of a high (ovulatory) dose of hCG (for example 4,000 to 11,000 IU hCG, e.g. 5,000 IU hCG, 10,000 IU hCG etc.; or 150 to 350 microgram recombinant hCG, for example 250 microgram recombinant hCG) to induce final follicular maturation.

It will be appreciated that the product may be for dosing at frequencies more (or less) than daily, in which case the relevant doses will be equivalent to the (daily) doses specified herein.

Herein the term "treatment of infertility" includes treatment of infertility by controlled ovarian stimulation (COS) or methods which include a step or stage of controlled ovarian stimulation (COS), for example Intra Uterine Insemination (IUI), in vitro fertilisation (IVF), or intracytoplasmic sperm injection (ICSI). The term "treatment of infertility" includes treatment of infertility by ovulation induction (01) or by methods which include a step or stage of ovulation induction (01). The term "treatment of infertility" includes treatment of infertility in a subject having tubal or unexplained infertility, including treatment of infertility in a subject having endometriosis, for example stage I or stage II endometriosis, and/or in a subject having anovulatory infertility, for example WHO type II anovulatory infertility, and/or in a subject with a partner with male factor infertility. The product (or composition) may be for (use in) the treatment of infertility (and/or for controlled ovarian stimulation) in a subject having endometriosis, for example in a subject having stage I or stage II endometriosis, as defined by The American Society for Reproductive Medicine (ASRM) classification system for the various stages of endometriosis, (stage IV most severe; stage I least severe) [American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril 1997; 67, 817 821.].

The product (composition) may be for (use in) the treatment of infertility (and/or for controlled ovarian stimulation) in a subject having normal serum FSH level of 1 to 16 IU/L, for example 1 to 12 IU/L, in the early follicular phase.

The product (composition) may be for (use in) the treatment of infertility (and/or for controlled ovarian stimulation) in a subject aged 18 to 42 years, for example 25 to 37 years. The product may be for (use in) the treatment of infertility (and/or for controlled ovarian stimulation) in a subject having BMI>1 and BMI<35 kg/m^2, for example a subject having BMI>18 and BMI<25 kg/m^2, for example a subject having BMI>20 and BMI<25 kg/m^2.

The rFSH may preferably include 27-33%, for example 30-32%, tri-sialylated glycan structures. The rFSH may preferably include 24-33%, for example 26-30%, di-sialylated glycan structures. The rFSH may preferably include 12-21%, for example 15-17%, mono-sialylated glycan structures. The rFSH preferably includes mono-sialylated, di-sialylated, tri-sialylated and tetra-sialylated glycan structures with relative amounts as follows: 15 to 17% mono-sialylated; 26-30% di-sialylated; 27-33% (e.g. 29 to 32%, e.g 30-32%, e.g 30 to 31%) tri-sialylated and 17-23% tetra-sialylated (e.g. as shown by WAX analysis of charged glycans, as set out in the Examples). The rFSH may include from 0 to 7%, for example 0.1 to 7%, for example 3 to 6%, for example 5 to 6%, neutral sialylated structures. The FSH comprises glycans (attached to the FSH glycoproteins). Herein, terminology such as "X % mono-sialylated", "X % di-sialylated", "X % tri-sialylated" or "X % tetra-sialylated" refers to the number of glycan structures on FSH which are mono-, di, tri or tetra sialylated (respectively), expressed as a percentage (X %) of the total number of glycan structures on the FSH which are sialylated in any way (carry sialic acid). Thus, the phrase "27-33% tri-sialylated glycan structures" means that, of the total number of glycan structures on FSH which carry sialic acid residues (that is, are sialylated), 27 to 33% of these glycan structures are tri sialylated (carry three sialic acid residues).

The rFSH may have a sialic acid content [expressed in terms of a ratio of moles of sialic acid to moles of protein] of 6 mol/mol or greater, for example between 6 mol/mol and 15 mol/mol, e.g between 8 mol/mol and 14 mol/mol, for example between 10 mol/mol and 14 mol/mol, e.g between 11 mol/mol and 14 mol/mol, e.g between 12 mol/mol and 14 mol/mol, e.g. between 12 mol/mol and 13 mol/mol. The rFSH may be produced or expressed in a human cell line.

The FSH (rFSH) for use according to the invention may have 1% to 99% of the total sialylation being α2,3-sialylation. The rFSH may have 10% or more of the total sialylation being α2,3-sialylation. For example, 20, 30, 40, 50, 60, 70, 80 or 90% or more of the total sialylation may be α2,3-sialylation. The rFSH may preferably include α2,3-sialylation in an amount which is from 50 to 70% of the total sialylation, for example from 60 to 69% of the total sialylation, for example from 63 to 67%, for example around 65% of the total sialylation. The FSH (rFSH) for use according to the invention may have 1% to 99% of the total sialylation being α2,6-sialylation. The rFSH (or rFSH preparation) of the invention may have 5% or more, for example 5% to 99%, of the total sialylation being α2,6-sialylation. The rFSH may have 50% or less of the total sialylation being α2,6-sialylation. The rFSH may preferably include α2,6-sialylation in an amount which is from 25 to 50% of the total sialylation, for example from 30 to 50% of the total sialylation, for example from 31 to 38%, for example around 35% of the total sialylation. By sialylation, it is meant the amount of sialic residues present on the FSH carbohydrate structures. α2,3-sialylation means sialylation at the 2,3 position (as is well known in the art) and α2,6 sialylation at the 2,6 position (also well known in the art). Thus "% of the total sialylation may be a 2,3 sialylation" refers to the % of the total number of sialic acid residues present in the FSH which are sialylated in the 2,3 position. The term "% of the total sialylation being α2,6-sialylation" refers to the % of the total number of sialic acid residues present in the FSH which are sialylated in the 2,6 position.

The rFSH may have a sialic acid content (amount of sialylation per FSH molecule) of (based on the mass of protein, rather than the mass of protein plus carbohydrate) of 6% or greater (e.g. between 6% and 15%, e.g. between 7% and 13%, e.g. between 8% and 12%, e.g. between 11% and 15%, e.g. between 12% and 14%) by mass.

The rFSH may be rFSH or a rFSH preparation in which 16% or fewer (e.g. 0.1 to 16%) of the glycans comprise (e.g. carry) bisecting N-acetylglucosamine (bisecting GlcNAc or bisGlcNAc). Preferably the rFSH (or rFSH preparation) is an rFSH or rFSH preparation in which 8 to 14.5% of the glycans comprise (e.g. carry) a bisecting N-acetylglucosamine (bisecting GlcNAc or bisGlcNAc).

It will be understood that FSH comprises glycans attached to the FSH glycoproteins. It will also be understood that 100% of the glycans refers to or means all of the glycans attached to the FSH glycoproteins. Thus, herein, the terminology "8 to 14.5% of the glycans comprise (carry) bisecting N-acetylglucosamine" means that 8 to 14.5% of the total number of glycans attached to the FSH glycoproteins include/carry bisecting N-acetylglucosamine; "16% or fewer of the glycans comprise (carry) bisecting N-acetylglucosamine" means that 16% or fewer of the total number of glycans attached to the FSH glycoproteins include/carry bisecting N-acetylglucosamine, and so on.

The applicants have found that recombinant FSH (rFSH preparations; rFSH compositions) in which 16% or fewer (e.g. 8 to 14.5%) of the glycans comprised in the FSH glycoproteins carry bisecting GlcNac may have advanta-

geous pharmacokinetic properties. It is believed the advantageous properties may arise because the amount of glycans which carry bisecting GlcNac is similar to that in the human urinary derived product Bravelle, which is rather less than that of other recombinant FSH preparations such as those disclosed in WO2012/017058.

The rFSH (or rFSH preparation) may be an rFSH or rFSH preparation in which 20% or more of the glycans comprise (e.g. carry)N-Acetylgalactosamine (GalNAc), for example in which 20% or more of the glycans comprise (e.g. carry) a terminal GalNAc. Preferably the rFSH (or rFSH preparation) is an FSH or FSH preparation in which the 40 to 55%, for example 42% to 52%, of the glycans comprise (e.g. carry) GalNAc. Preferably the rFSH (or rFSH preparation) is an FSH or FSH preparation in which the 40 to 55%, for example 42% to 52%, of the glycans comprise (e.g. carry) terminal GalNAc.

It will be understood that FSH comprises glycans attached to the FSH glycoproteins. It will also be understood that 100% of the glycans refers to or means all of the glycans attached to the FSH glycoproteins. Thus, herein, the terminology "wherein 20% or more of the glycans comprise (e.g. carry) GalNAc" means that 20% or more of the total number of glycans attached to the FSH glycoproteins include/carry N-Acetylgalactosamine (GalNAc); "40 to 55%, for example 42% to 52%, of the glycans comprise (e.g. carry) terminal GalNAc" means that 40 to 55%, for example 42% to 52%, of the total number of glycans attached to the FSH glycoproteins include/carry terminal GalNAc, and so on.

It appears that the availability of the α2,6-linkage increases the number of tetra sialylated structures, compared to CHO cell derived products which have only the α2,3-linkage available. The applicants have also found that their rFSH is distinguished over other approved products because of the sugar composition: it includes, or may include, a specific amount of GalNac. This may be linked to tetrasialylation and potency because the 2,6-sialylation is associated with GalNac. In other words, the present applicants have developed an rFSH product which includes specific characteristics (2,6-linker sites, GalNac) which provide rFSH with high degree of sialylation, which appears to lead to improved potency in vivo.

The rFSH (or rFSH preparation) may have 16 to 24% of the glycans comprising (e.g. terminal) 1 fucose-lewis, for example 16.5 to 18% of the glycans comprising (e.g. terminal) 1 fucose-lewis. The rFSH (or rFSH preparation) may have 1.5 to 4.5%, for example 2 to 4%, for example 3.7%, of the glycans comprising (e.g. terminal) 2 fucose-lewis. The content of fucose-lewis may have an effect on potency.

The rFSH may be produced or expressed in a human cell line, for example a Per.C6 cell line, a HEK293 cell line, a HT1080 cell line etc. This may simplify (and render more efficient) the production method because manipulation and control of e.g. the cell growth medium to retain sialylation may be less critical than with known processes. The method may also be more efficient because there is little basic rFSH produced compared to production of known rFSH products; more acidic rFSH is produced and separation/removal of basic FSH is less problematic. The rFSH may be produced or expressed in a PER.C6® cell line, a PER.C6® derived cell line or a modified PER.C6® cell line. rFSH which is produced or expressed in a human cell line (e.g. PER.C6® cell line, HEK293 cell line, HT1080 cell line etc.) will include some α2,6-linked sialic acids (α2,6 sialylation) provided by endogenous sialyl transferase activity [of the cell line] and will include some α2,3-linked sialic acids (α2,3 sialylation) provided by endogenous sialyl transferase activity. The cell line may be modified using α2,3-sialyltransferase. The cell line may be modified using α2,6-sialyltransferase. Alternatively or additionally, the rFSH may include α2,6-linked sialic acids (α2,6 sialylation) provided by endogenous sialyl transferase activity [of the cell line]. Herein, the term "human derived recombinant FSH" means recombinant FSH which is produced or expressed in a human cell line (e.g. recombinant FSH made by engineering a human cell line).

The rFSH may be produced using α2,3- and/or α2,6-sialyltransferase. In an example, rFSH is produced using α2,3-sialyltransferase. The rFSH may include α2,6-linked sialic acids (α2,6 sialylation) provided by endogenous sialyl transferase activity.

The product may be a pharmaceutical composition. The pharmaceutical composition is for the treatment of infertility. The treatment of infertility may comprise assisted reproductive technologies (ART), ovulation induction or intrauterine insemination (IUI). The pharmaceutical composition may be used, for example, in medical indications where known FSH preparations are used.

The product or composition can be formulated into well-known compositions for any route of drug administration, e.g. oral, rectal, parenteral, transdermal (e.g. patch technology), intravenous, intramuscular, subcutaneous, intrasusternal, intravaginal, intraperitoneal, local (powders, ointments or drops) or as a buccal or nasal spray. A typical composition comprises a pharmaceutically acceptable carrier, such as aqueous solution, non toxic excipients, including salts and preservatives, buffers and the like, as described in Remington's Pharmaceutical Sciences fifteenth edition (Matt Publishing Company, 1975), at pages 1405 to 1412 and 1461-87, and the national formulary XIV fourteenth edition (American Pharmaceutical Association, 1975), among others.

Examples of suitable aqueous and non-aqueous pharmaceutical carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectible organic esters such as ethyl oleate. The compositions of the present invention also can contain additives such as but not limited to preservatives, wetting agents, emulsifying agents, surfactants and dispersing agents. Antibacterial and antifungal agents can be included to prevent growth of microbes and includes, for example, m-cresol, benzyl alcohol, paraben, chlorobutanol, phenol, sorbic acid, and the like. If a preservative is included, benzyl alcohol, phenol and/or m-cresol are preferred; however, the preservative is by no means limited to these examples. Furthermore, it may be desirable to include isotonic agents such as sugars, sodium chloride, and the like. The product or composition may further comprise a salt comprising a pharmaceutically acceptable alkali metal cation selected from the group consisting of Na^+- or K^+-salts, or a combination thereof. Preferably the salt is a Na+-salt, for example NaCl or Na_2SO_4.

Preferably the product or composition comprises recombinant FSH and one or more of Polysorbate 20, L-methionine, phenol, disodium sulphate and sodium phosphate buffer.

In some cases, to effect prolonged action it is desirable to slow the absorption of FSH (and other active ingredients, if present) from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of FSH then depends upon its rate of dissolution which, in turn, can depend upon crystal size and

crystalline form. Alternatively, delayed absorption of a parenterally administered FSH combination form is accomplished by dissolving or suspending the FSH combination in an oil vehicle. Injectable depot forms can be made by forming microencapsule matrices of the FSH (and other agents, if present) in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of FSH to polymer and the nature of the particular polymer employed, the rate of FSH release can be controlled. Examples of other biodegradable polymers include polyvinylpyrrolidone, poly (orthoesters), poly(anhydrides) etc. Depot injectable formulations are also prepared by entrapping the FSH in liposomes or microemulsions which are compatible with body tissues.

Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use. Injectable formulations can be supplied in any suitable container, e.g. vial, pre-filled syringe, injection cartridges, and the like.

The product or composition may be formulated for single use or for multiple use (multiple dose). If the product or composition is formulated for multiple use, it is preferred that a preservative is included. If a preservative is included, benzyl alcohol, phenol and/or m-cresol are preferred; however, the preservative is by no means limited to these examples. The single use or multiple use formulated product or composition may further comprise a salt comprising a pharmaceutically acceptable alkali metal cation selected from the group consisting of Na^+- or K^+-salts, or a combination thereof. Preferably the salt is a Na+-salt, for example NaCl or Na_2SO_4.

The product or composition may be included in a container such as a vial, prefilled cartridge (e.g. for single administration or multiple use) or an injection device such as a "pen" for e.g. administration of multiple doses.

The product or composition may be a formulation (e.g. injectable formulation) including FSH (optionally with hCG, LH, LH activity etc.) The LH activity, if present, may originate from LH or human chorionic gonadotropin, hCG. If there is more than one active ingredient (i.e. FSH and e.g. hCG or LH) these may be suitable for administration separately or together. If administered separately, administration can be sequential. The product can be supplied in any appropriate package. For example, a product can include a number of containers (e.g. pre-filled syringes or vials) containing either FSH or hCG, or a combination (or combination) of both FSH and hCG. The hCG may be recombinant hCG or urinary hCG. If the product includes a number of containers (e.g. pre-filled syringes or vials) containing FSH, e.g. recombinant FSH, each container may include the same amount of FSH. One or more containers may include different amounts of FSH. The syringes or vials may be packaged in a blister package or other means to maintain sterility. Any product can optionally contain instructions for using the FSH (and e.g. hCG if present) formulations. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted in accordance with routine practice in this field. See GOODMAN and GILMAN's THE PHARMACOLOGICAL BASIS FOR THERAPEUTICES, 7^{th} ed. In a preferred embodiment, the compositions of the invention are supplied as compositions for parenteral administration. General methods for the preparation of the parenteral formulations are known in the art and are described in REMINGTON; THE SCIENCE AND PRACTICE OF PHARMACY, supra, at pages 780-820. The parenteral compositions can be supplied in liquid formulation or as a solid which will be mixed with a sterile injectable medium just prior to administration. In an especially preferred embodiment, the parenteral compositions are supplied in dosage unit form for ease of administration and uniformity of dosage.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) measuring the serum AMH level of a subject; and (b) administration to the subject a dose of, or a dose equivalent to, 1-24 μg, for example 2-24 μg, for example 2 to 15 μg, human derived recombinant FSH. Preferably the dose is, or is equivalent to, 4.5 to 12.5 μg, for example 5 to 12.5 μg, for example 6 to 12.5 μg, for example 6.3 to 12 μg, human derived recombinant FSH.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) determining (e.g. measuring) the serum AMH level of a subject; and (b) administering a (e.g. daily) dose of, or dose equivalent to, 9 to 14 μg, for example 11 to 13 μg, for example 12 μg human derived recombinant follicle stimulating hormone (FSH) to a (the) subject having serum AMH level of <15 pmol/L (e.g. 0.05 pmol/L to 14.9 pmol/L). Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The dose provides an effective response while minimising risk of OHSS.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) determining (e.g. measuring) the serum AMH level of a subject; and (b) administering a (e.g. daily) dose of, or dose equivalent to, 5 to 12.5 μg human derived recombinant follicle stimulating hormone (FSH) to a (the) subject having serum AMH level of ≥15 pmol/L. The (e.g. daily) dose may be, or be equivalent to, 6 to 10 μg human derived recombinant follicle stimulating hormone (FSH). Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The dose provides an effective response while minimising risk of OHSS.

In one embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 5 to 12 μg, for example 7 to 12 μg, for example 8.7 to 10 μg, human derived recombinant FSH (preferably 9 to 10 μg human derived recombinant FSH) to a (the) subject having serum AMH level of 15 to 24.9 pmol/L In another embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 5 to 12 μg human derived recombinant FSH (for example 7 to 12 μg, for example 6 to 9 μg, for example 7 to 8 μg, for example 7.3 to 8 μg human derived recombinant FSH) to a (the) subject having serum AMH level of 25 to 34.9 pmol/L. In another embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 5 to 11 μg human derived recombinant FSH (for example 6 to 7 μg, for example 6.3 to 7 μg, human derived recombinant FSH) to a (the) subject having serum AMH level of ≥35 pmol/L.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) determining (e.g. measuring) the serum AMH level of a subject; and (b) administering a (e.g. daily) dose of, or dose equivalent to, 0.09 to 0.19 μg (for example 0.09 to 0.17 μg) human derived recombinant FSH per kg bodyweight of the subject, wherein the subject has serum AMH level of ≥15 pmol/L. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The dose provides an effective response while minimising risk of OHSS.

In one embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 0.14 to 0.19 μg human derived recombinant FSH (preferably 0.15 to 0.16 μg human derived recombinant FSH) per kg bodyweight of the subject, the subject having serum AMH level of 15 to 24.9 pmol/L. In another embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 0.11 to 0.14 μg human derived recombinant FSH (preferably 0.12 to 0.13 μg human derived recombinant FSH) per kg bodyweight of the subject, the subject having serum AMH level of 25 to 34.9 pmol/L. In another embodiment, the method includes a step of administering a (e.g. daily) dose of, or dose equivalent to, 0.10 to 0.11 μg human derived recombinant FSH per kg bodyweight of the subject, the subject having serum AMH level of 35 pmol/L. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. These doses provide an effective response while minimising risk of OHSS.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) determining (e.g. measuring) the serum AMH level of a subject; and (b) administering a (e.g. daily) dose of, or dose equivalent to, 0.15 to 0.21 μg (for example 0.19 to 0.21 μg) human derived recombinant FSH per kg bodyweight of the subject, wherein the subject has serum AMH level of <15 pmol/L.

The administration preferably comprises a daily dose of, or a daily dose equivalent to, the amount of FSH defined above and in the claims. The (daily) dose may be an initial dose (it may be reduced, increased, or maintained during the treatment).

The method may be a method of treatment of infertility in the patient's (subject's) first stimulation protocol. It will be appreciated that for further stimulation cycles, the doses may be adjusted according to actual ovarian response in the first cycle.

According to the present invention in a further aspect there is provided a method of treatment of infertility comprising: (a) determining (e.g. measuring) the serum AMH level of a subject;

and (b) if the subject has serum AMH level of <15 pmol/L (e.g. 0.05 pmol/L to 14.9 pmol/L), administering to the subject a dose of, or dose equivalent to, 10 to 14 μg, for example 11 to 13 μg, for example 12 μg, human derived recombinant follicle stimulating hormone (FSH); or

if the subject has serum AMH level of 15 to 24.9 pmol/L, administering to the subject a dose of, or dose equivalent to, 0.14 to 0.19 μg human derived recombinant FSH (preferably 0.15 to 0.16 μg human derived recombinant FSH) per kg bodyweight of the subject; or

if the subject has serum AMH level of 25 to 34.9 pmol/L pmol/L, administering to the subject a dose of, or dose equivalent to, 0.11 to 0.14 μg human derived recombinant FSH (preferably 0.12 to 0.13 μg human derived recombinant FSH) per kg bodyweight of the subject; or

if the subject has serum AMH level of ≥35 pmol/L pmol/L, administering to the subject a dose of, or dose equivalent to, 0.10 to 0.11 μg human derived recombinant FSH per kg bodyweight of the subject.

For a patient (subject) having serum AMH of 5.0-14.9 pmol/L, a dose of, or dose equivalent to, 6 to 18 μg, for example 8 to 11 μg, for example 8.5 to 10.2 μg human derived recombinant FSH may be administered. For a patient (subject) having serum AMH 15.0-29.9 pmol/L, a dose of, or a dose equivalent to, 4.8 to 15 μg, for example 6 to 9 μg, for example 6.8 to 8.5 μg human derived recombinant FSH may be administered. For a patient (subject) having serum AMH 30-44.9 pmol/L, a dose of, or a dose equivalent to, 3.6 to 12 μg, for example 4 to 7 μg, for example 5.1 to 6.8 μg human derived recombinant FSH may be administered. For a patient (subject) having serum AMH 45 pmol/L or greater, a dose of, or a dose equivalent to, 2 to 9 μg, for example 2.4 to 9 μg (for example 3.4 to 5.1 μg) or 2 to 5 μg human derived recombinant FSH may be administered. For a patient (subject) having serum AMH of 5 pmol/L or less, a dose of, or a dose equivalent to 7.2 to 24 μg, for example 10 to 15 μg for example 10.2 to 13.6 μg, human derived recombinant FSH may be administered. In some examples, a dose of, or dose equivalent to, 4.8 to 18 μg, for example 6 to 11 μg, for example 6.8 to 10.2 μg human derived recombinant FSH is administered. Preferably the FSH is a recombinant FSH ("rFSH" or "recFSH"). Preferably the FSH is a human cell line derived recombinant FSH. The administration preferably comprises a daily dose of, or a daily dose equivalent to, the amount of FSH defined above and in the claims. The (daily) dose may be an initial dose (it may be reduced, increased, or maintained during the treatment.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will now be described in more detail with reference to the attached drawings in which:

FIG. **1** shows a plasmid map of the pFSHalpha/beta expression vector;

FIG. **2** shows the α2,3-sialyltransferase (ST3GAL4) expression vector;

FIG. **3** shows the α2,6-sialyltransferase (ST6GAL1) expression vector;

FIG. **4** shows % abundance sialic acid distribution of examples of recombinant FSH produced by PER.C6® cells stably expressing FSH after engineering with α2,3-sialyltransferase;

FIG. **5** shows % abundance of glycan charge distribution of examples of recombinant FSH produced by PER.C6® cells stably expressing FSH after engineering with α2,3-sialyltransferase;

FIG. **6** shows a comparison of concentration of inhibin-B following administration of 225 IU Gonal f (bottom line, dotted line) and 225 IU of the Example (top line, full line) of Invention;

FIG. **7** shows the effect of body weight on oocytes retrieved in the low AMH treatment group (Example 10, 10A); and

FIG. **8** shows the effect of Body weight on oocytes retrieved in the high AMH treatment group

SEQUENCE SELECTION

Human FSH

The coding region of the gene for the FSH alpha polypeptide was used to according to Fiddes and Goodman. (1981). The sequence is banked as AH007338 and at the time of construction there were no other variants of this protein sequence. The sequence is referred herein as SEQ ID NO:1.

The coding region of the gene for FSH beta polypeptide was used according to Keene et al (1989). The sequence is banked as NM_000510 and at the time of construction there were no other variants of this protein sequence. The sequence is referred herein as SEQ ID NO: 2

Sialyltransferase

α2,3-Sialyltransferase—The coding region of the gene for beta-galactoside alpha-2,3-sialyltransferase 4 (α2,3-

sialyltransferase, ST3GAL4) was used according to Kitagawa and Paulson (1994). The sequence is banked as L23767 and referred herein as SEQ ID NO: 3.

α2,6-Sialyltransferase—The coding region of the gene for beta-galactosamide alpha-2,6-sialyltransferase 1 (α2,6-sialyltransferase, ST6GAL1) was used according to Grundmann et al. (1990). The sequence is banked as NM_003032 and referred herein as SEQ ID NO: 4.

EXAMPLES

Example 1 Construction of the FSH Expression Vector

The coding sequence of FSH alpha polypeptide (AH007338, SEQ ID NO: 1) and FSH beta polypeptide (NM_003032, SEQ ID NO: 2) were amplified by PCR using the primer combinations FSHa-fw and FSHa-rev and FSHb-fw and FSHb-rec respectively.

```
FSHa-fw
                                        (SEQ ID NO: 9)
5'-CCAGGATCCGCCACCATGGATTACTACAGAAAAATATGC-3'

FSHa-rev
                                        (SEQ ID NO: 10)
5'-GGATGGCTAGCTTAAGATTTGTGATAATAAC-3'

FSHb-fw
                                        (SEQ ID NO: 11)
5'-CCAGGCGCGCCACCATGAAGACACTCCAGTTTTTC-3'

FSHb-rev
                                        (SEQ ID NO: 12)
5'-CCGGGTTAACTTATTATTCTTTCATTTCACCAAAGG-3'
```

The resulting amplified FSH beta DNA was digested with the restriction enzymes AscI and HpaI and inserted into the AscI and HpaI sites on the CMV driven mammalian expression vector carrying a neomycin selection marker. Similarly the FSH alpha DNA was digested with BamHI and NheI and inserted into the sites BamHI and NheI on the expression vector already containing the FSH beta polypeptide DNA.

The vector DNA was used to transform the DH5a strain of *E. coli*. Colonies were picked for amplification. Colonies containing the vector containing both FSH alpha and beta were selected for sequencing and all contained the correct sequences according to SEQ ID NO: 1 and SEQ ID NO: 2. Plasmid pFSH A+B #17 was selected for transfection (FIG. **1**).

Example 2 Construction of the ST3 Expression Vector

The coding sequence of beta-galactoside alpha-2,3-sialyltransferase 4 (ST3, L23767, SEQ ID NO: 3) was amplified by PCR using the primer combination 2,3STfw and 2,3STrev.

```
2,3STfw
                                        (SEQ ID NO: 13)
5'-CCAGGATCCGCCACCATGTGTCCTGCAGGCTGGAAGC-3'

2, 3STrev
                                        (SEQ ID NO: 14)
5'-TTTTTTTCTTAAGTCAGAAGGACGTGAGGTTCTTG-3'
```

The resulting amplified ST3 DNA was digested with the restriction enzymes BamHI and AflII and inserted into the BamHI and AflII sites on the CMV driven mammalian expression vector carrying a hygromycin resistance marker. The vector was amplified as previously described and sequenced. Clone pST3 #1 (FIG. **2**) contained the correct sequence according SEQ ID NO: 3 and was selected for transfection.

Example 3 Construction of the ST6 Expression Vector

The coding sequence of beta-galactosamide alpha-2,6-sialyltransferase 1 (ST6, NM_003032, SEQ ID NO: 4) was amplified by PCR using the primer combination 2,6STfw and 2,6STrev.

```
2,6STfw
                                        (SEQ ID NO: 15)
5'-CCAGGATCCGCCACCATGATTCACACCAACCTGAAG-3'
2,6STrev
                                        (SEQ ID NO: 16)
5'-TTTTTTTCTTAAGTTAGCAGTGAATGGTCCGG-3'
```

The resulting amplified ST6 DNA was digested with the restriction enzymes BamHI and AflII and inserted into the BamHI and AflII sites on the CMV driven mammalian expression vector carrying a hygromycin resistance marker. The vector was amplified as previously described and sequenced. Clone pST6 #11 (FIG. **3**) contained the correct sequence according SEQ ID NO: 4 and was selected for transfection.

Example 4 Stable Expression of pFSH α+β in PER.C6® Cells. Transfection Isolation and Screening of Clones

PER.C6® clones producing FSH were generated by expressing both polypeptide chains of FSH from a single plasmid (see Example 1).

To obtain stable clones a liposome based transfection agent with the pFSH α+β construct. Stable clones were selected in VPRO supplemented with 10% FCS and containing G418. Three weeks after transfection G418 resistant clones grew out. Clones were selected for isolation. The isolated clones were cultured in selection medium until 70-80% confluent. Supernatants were assayed for FSH protein content using an FSH selective ELISA and pharmacological activity at the FSH receptor in cloned cell line, using a cAMP accumulation assay. Clones expressing functional protein were progressed for culture expansion to 24 well, 6 well and T80 flasks.

Studies to determine productivity and quality of the material from seven clones were initiated in T80 flasks to generate sufficient material. Cells were cultured in supplemented media as previously described for 7 days and the supernatant harvested. Productivity was determined using the FSH selective ELISA. The isoelectric profile of the material was determined by Isoelectric focusing (IEF), by methods known in the art. Clones with sufficient productivity and quality were selected for sialyltransferase engineering.

Example 5 Level of Sialylation is Increased in Cells that Over Express α2,3-Sialyltransferase. Stable Expression of pST3 in FSH Expressing PER.C6® Cells; Transfection Isolation and Screening of Clones

PER.C6® clones producing highly sialylated FSH were generated by expressing α2,3 sialyltransferase from separate

plasmids (Example 2) in PER.C6® cells already expressing both polypeptide chains of FSH (from Example 4). Clones produced from PER.C6® cells as set out in Example 4 were selected for their characteristics including productivity, good growth profile, production of functional protein, and produced FSH which included some sialylation. Stable clones were generated as previously described in Example 4. Clones were isolated, expanded and assayed. The α2,3-sialyltransferase clones were adapted to serum free media and suspension conditions.

As before, clones were assayed using a FSH selective ELISA, functional response in an FSH receptor cell line, IEF, metabolic clearance rate and Steelman Pohley analysis. Results were compared to a commercially available recombinant FSH (Gonal-f, Serono) and the parental FSH PER.C6® cell lines. FSH produced by most of the clones has significantly improved sialylation (i.e. on average more FSH isoforms with high numbers of sialic acids) compared to FSH expressed without α2,3-sialyltransferase. In conclusion expression of FSH together with sialyltransferase in PER.C6® cells resulted in increased levels of sialylated FSH compared to cells expressing FSH only.

Example 6 Production and Purification Overview

A procedure was developed to produce FSH in PER.C6® cells that were cultured in suspension in serum free medium. The procedure is described below and was applied to several FSH-producing PER.C6® cell lines.

FSH from α2,3-clone (Example 5) was prepared using a using a modification of the method described by Lowry et al. (1976).

For the production of PER.C6®-FSH, the cell lines were adapted to a serum-free medium, i.e., Excell 525 (JRH Biosciences). The cells were first cultured to form a 70%-90% confluent monolayer in a T80 culture flask. On passage the cells were re-suspended in the serum free medium, Excell 525+4 mM L-Glutamine, to a cell density of 0.3×10^6 cells/ml. A 25 ml cell suspension was put in a 250 ml shaker flask and shaken at 100 rpm at 37° C. at 5% CO_2. After reaching a cell density of $>1\times10^6$ cells/ml, the cells were sub-cultured to a cell density of 0.2 or 0.3×10^6 cells/ml and further cultured in shaker flasks at 37° C., 5% CO_2 and 100 rpm.

For the production of FSH, the cells were transferred to a serum-free production medium, i.e., VPRO (JRH Biosciences), which supports the growth of PER.C6® cells to very high cell densities (usually $>10^7$ cells/ml in a batch culture). The cells were first cultured to $>1\times10^6$ cells/ml in Excell 525, then spun down for 5 min at 1000 rpm and subsequently suspended in VPRO medium+6 mM L-glutamine to a density of 1×10^6 cells/ml. The cells were then cultured in a shaker flask for 7-10 days at 37° C., 5% CO_2 and 100 rpm. During this period, the cells grew to a density of $>10^7$ cells/ml. The culture medium was harvested after the cell viability started to decline. The cells were spun down for 5 min at 1000 rpm and the supernatant was used for the quantification and purification of FSH. The concentration of FSH was determined using ELISA (DRG EIA 1288).

Thereafter, purification of FSH was carried out using a modification of the method described by Lowry et al. (1976). Purification using charge selective chromatography was carried out to enrich the highly sialylated forms by methods well known in the art.

During all chromatographic procedures, enrichment of the sialylated forms of FSH as claimed herein was confirmed by RIA (DRG EIA 1288) and/or IEF.

Example 7 Quantification of Relative Amounts of α2,3 and α2,6 Sialic Acid

The relative percentage amounts of α2,3 and α2,6 sialic acid on purified rFSH (Example 6) were measured using known techniques.

N-Glycans were released from the samples using PNGase F under denaturative conditions and then labelled with 2-aminobenzamide. Released glycan forms were then separated and analysed by Weak Anion Exchange (WAX) column for determination of charge distribution. Labelled glycans treated with 2, 3, 6, 8 sialidase for determination of total sialic acid and 2,3 sialidase for determination of 2,3 sialic acid, were further analyzed by wax column.

The relative percentages of the charged glycans were calculated from structures present in the undigested and digested glycan pools and are shown in FIG. **4** (for 8 samples). These were found to be in the ranges 50%-70% (e.g. about 60% or 65%) for α2,3 sialylation and 28 to 50%, generally 30 to 35% (e.g. about 31% or 35%), for α2,6 sialylation.

Example 8 Quantification of Relative Amounts Mono, Di, Tri and Tetra Sialylated Glycan Structures

The relative percentage amounts of mono, di, tri and tetra sialylated structures on glycans extracted from purified rFSH (Example 6) were measured using known techniques.

N Glycans were released from the samples using PNGase F under denaturative conditions and then were labeled with 2-aminobenzamide. Glycans were released from the samples using PNGase F under denaturative conditions and then labeled with 2-aminobenzamide. Released glycan forms were then separated and analysed by Weak Anion Exchange (WAX) column for determination of sialylation distribution. The relative amounts of neutral, mono-sialylated, di-sialylated, tri-sialylated and tetra-sialylated structures are shown in FIG. **5** (for the 8 samples shown in FIG. **4**).

The rFSH includes neutral, mono-sialylated, di-sialylated, tri-sialylated and tetra-sialylated glycan structures with relative amounts as follows: neutral 5-6%; 15-17% mono-sialylated; 26-30% di-sialylated; 30-32% tri-sialylated and 17-23% tetra-sialylated.

Example 8a

The relative percentage amounts of α2,6 sialic acid on purified rFSH extracted from nine samples of purified rFSH (produced by the methods of Example 6) were measured using known techniques.

N-Glycans were released from the samples using PNGase F under denaturative conditions and then labelled with 2-aminobenzamide. Released glycan forms were then separated and analysed by Weak Anion Exchange (WAX) column for determination of charge distribution. Labelled glycans treated with 2, 3, 6, 8 sialidase for determination of total sialic acid and 2,3 sialidase for determination of 2,3 sialic acid, were further analyzed by wax column (see Example 8). The analysis allows calculation of α2,6 sialic acid.

The relative percentages of the charged glycans were calculated from structures present in the undigested and digested glycan pools and are shown in the following Table.

These were found to be in the ranges 25 to 50%, generally 30 to 35% for α2,6 sialylation.

The relative percentage amounts of bisecting GlcNac, GalNac and 1-Fucose Lewis on glycans extracted from the nine samples of purified rFSH (produced by the methods of Example 6) were measured using known techniques. N-Glycans were released from the glycoprotrein using PNGase F and labeled with 2-aminobenzamide (2AB). The analysis was done by two dimensional (2D) HPLC analysis in combination with enzymatic degradation of the glycans. For verification, the glycans were analyzed by MALDI-MS The relative amounts of alpha 2,6-sialic acid and the terminal residues are shown in the following table, together with those for Gonal F (CHO cell derived recombinant FSH) and Bravelle (human urinary FSH).

Sample	Ref. O abundance %	Ref. N abundance %	I-1 abundance %	I-2 abundance %	I-3 abundance %	II abundance %	II abundance %
2,6 sialic acid	27.7	34.9	26.2	30.1	31.1	28.3	30.4
1GalNAc	51	44.6	50.7	44.7	49	47.6	45.3
Bisecting GlcNAc	10	12.4	10.2	8.9	8.7	11.8	11.4
1 Fucose Lewis	21.1	16.7	23.3	16.1	20.3	18.1	17.9
2 Fucose Lewis	4	4.1	4.3	1.9	3.1	4.2	3.8

Sample	III-1 abundance %	III-2 abundance %	Average abundance %	Gonal F abundance %	Bravelle abundance %
2,6 sialic acid	35	33	30.7	0	55.4
1GalNAc	46.4	44.9	47.1	0	11.3
Bisecting GlcNAc	10.6	13.9	10.9	55	14
1 Fucose Lewis	18.7	19.0	19.0	3.1[1]	2.2
2 Fucose Lewis	3.9	4.4	3.7	—	n.d.[2]

[1]Value of 3.1 is total ½ Fucose Lewis.
[2]Not determined.

It can be seen that the amount of GalNac in the FSH of the invention varies between about 44.9 and 51%, averaging about 47.1%.

It can be seen that the amount of bisecting GlcNac in the FSH of the invention varies between 8.7 and 13.9%, averaging approximately at 10.9%.

It can be seen that the amount of 1 Fucose Lewis in the FSH of the invention varies between 16.1 and 23.3%, averaging approximately at 19%.

It can be seen that the amount of 2 Fucose Lewis in the FSH of the invention varies between 1.9 and 4.4%, averaging approximately at 3.7%.

Example 9—a Multiple Dose Study Investigating the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Immunogenicity of FE 999049 in Comparison to GONAL-F

Study Population

A total of 48 (24 on each drug) healthy women received daily doses of 14.6 μg of FE 999049 (a composition according to the invention, produced according to Example 6) or 16.5 μg of Gonal-F for seven days.

Safety Results

Multiple dose administration of FE 999049 and GONAL-F was safe and generally well tolerated as assessed by Adverse Events (AEs), vital signs, ECG, clinical laboratory measurements, and physical examination. No serious adverse event or death occurred during the study.

Pharmacokinetic Results

Following the administration of FE 999049 and GONAL-F over 7 days, the FSH concentration values as assessed immediately prior to the next injection increased and seemed to reach a steady state level after 6-7 days. However the exposure (AUC and Cmax) of FE 999049 was 60% higher in comparison to Gonal-F.

Pharmacodynamic Results

The concentrations of inhibin-B (see FIG. **6**), oestradiol, and progesterone all increased subsequent to administration of FE 999049 and GONAL-F, however to a greater extent following administration of FE 999049 compared to GONAL-F. Both number and size distribution of follicles showed a greater response to FE 999049 compared to GONAL-F.

Example 9 demonstrates that FSH having a specific amount (17-23%) of tetra-sialylated glycan structures and e.g. specific amounts of α2,3 sialylation and α2,6 sialylation is markedly more potent then recombinant FSH products which are currently on the market.

Example 10—a Multiple Dose Study Investigating FE 999049 in Comparison to GONAL-F

The following describes a randomised, controlled, assessor-blind, parallel groups, multinational, multicentre trial assessing the dose-response relationship of FE 999049 in patients undergoing controlled ovarian stimulation for in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI). The patient population was 265 IVF patients aged between 18 to 37 years, with BMI 18.5 to 32.0 kg/m^2.

The trial was designed as a dose-response trial with number of oocytes retrieved as the primary endpoint. Secondary endpoints will explore the qualitative and quantitative impact of different doses of FE 999049 with regard to endocrine profile, follicular development, oocyte fertilisation, embryo quality and treatment efficiency (i.e. total gonadotropin consumption and duration of stimulation). The trial is designed to evaluate the efficacy of FE 999049 to establish pregnancy when used in controlled ovarian stimulation for IVF/ICSI cycles.

Subjects were assessed within 3 months prior to randomisation for compliance with the inclusion and exclusion criteria, including an anti-Müllerian hormone (AMH) assessment to increase homogeneity of the trial population in relation to ovarian response and minimise the number of potential poor and hyper-responders to the FE 999049 doses and GONAL-F dose used in the trial. The AMH assessment was measured using the AMH Gen-II enzyme linked immunosorbent assay kit (Beckman Coulter, Inc., Webster, Tex.). This assay can detect AMH concentrations greater than 0.57 pmol/L with a minimum limit of quantitation of 1.1 pmol/L.

On day 2-3 of their menstrual cycle, subjects were randomised in a 1:1:1:1:1:1 fashion to treatment with either 90 IU, 120 IU, 150 IU, 180 IU or 210 IU FE 999049 or 150 IU GONAL-F, and ovarian stimulation initiated. Randomisation was stratified according to AMH level at screening [5.0-14.9 pmol/L (low AMH) and 15.0 to 44.9 pmol/L (high AMH)).

Gonal-F is filled by mass (FbM) at FDA request; referring to μg dose is therefore appropriate. The Gonal-F label indicates 600 IU/44 μg, which indicates that 150 IU is 11 μg. However, there is some variation and the batch certificate for this trial indicated that 11.3 μg Gonal-F was equivalent to 150 IU. The FE999049 doses are presented by protein content (μg) rather than biological activity. Thus the doses of FE999049 were 5.2 μg (90 IU), 6.9 μg (120 IU), 8.6 μg (150 IU), 10.3 μg (180 IU) or 12.1 μg (210 IU).

The subject and dose distribution is set out as follows (data are number of subjects):

TABLE 1

	FE 999049					GONAL-F 11.3 (11)	
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg	μg	Total
Screened							334
Randomised and exposed	42	45	44	45	46	43	265
High AMH strata (15.0-44.9 pmol/L)	23	26	24	24	26	25	148 (56%)
Low AMH strata (5.0-14.9 pmol/L)	19	19	20	20	21	18	117 (44%)
Per-protocol	40	42	42	44	44	43	255

The daily dose level of FE 999049 or GONAL-F is fixed throughout the entire stimulation period. During stimulation, subjects are monitored on stimulation day 1, 4 and 6 and hereafter at least every second day. When 3 follicles of ≥15 mm are observed, visits are performed daily. Subjects are treated with FE 999049 or GONAL-F for a maximum of 16 days.

To prevent a premature LH surge, a GnRH antagonist (ganirelix acetate, ORGALUTRAN, MSD/Schering-Plough) may be initiated on stimulation day 6 at a daily dose of 0.25 mg and continued throughout the stimulation period. Triggering of final follicular maturation is done on the day when ≥3 follicles with a diameter ≥17 mm are observed. If there are <25 follicles with a diameter ≥12 mm, 250 μg recombinant hCG (choriogonadotropin alfa, OVITRELLE, Merck Serono/EMD Serono) is administered. If there are 25-35 follicles with a diameter ≥12 mm, 0.2 mg GnRH agonist (triptorelin acetate, DECAPEPTYL/GONAPEPTYL, Ferring Pharmaceuticals) is administered. In case of excessive ovarian response, defined as >35 follicles with a diameter ≥12 mm, the treatment is cancelled. In case of poor ovarian response, defined as <3 follicles with a diameter ≥10 mm observed on stimulation day 10, the cycle could be cancelled.

Oocyte retrieval takes place 36 h (±2 h) after triggering of final follicular maturation and the oocytes inseminated by IVF and/or ICSI. Fertilisation and embryo development are assessed from oocyte retrieval to the day of transfer. For subjects who underwent triggering of final follicular maturation with hCG, one blastocyst of the best quality available is transferred on day 5 after oocyte retrieval while remaining blastocysts are frozen. For subjects who undergo triggering of final follicular maturation with GnRH agonist, no embryo transfer takes place in the fresh cycle and blastocysts are instead frozen on day 5. Vaginal progesterone tablets (LUTINUS, Ferring Pharmaceuticals) 100 mg 3 times daily are provided for luteal phase support from the day after oocyte retrieval until the day of the clinical pregnancy visit. A βhCG test is performed 13-15 days after embryo transfer and clinical pregnancy will be confirmed by transvaginal ultrasound (TVU) 5-6 weeks after embryo transfer.

Results

The number of oocytes retrieved (primary endpoint) is shown in the following Table.

TABLE 2

	FE 999049					GONAL-F 11.3 (11)
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg	μg
Oocytes retrieved						
All	5.2 (3.3)	7.9 (5.9)	9.2 (4.6)	10.6 (7.0)	12.2 (5.9)	10.4 (5.2)
High AMH	5.9 (3.9)	9.1 (6.4)	10.6 (4.8)	13.6 (7.8)	14.4 (5.8)	12.4 (5.4)
Low AMH	4.5 (2.2)	6.3 (4.9)	7.4 (3.8)	6.9 (3.6)	9.4 (4.9)	7.8 (3.4)

Data are mean (SD)

The primary objective was met: a significant dose-response relationship was established for FE 999049 with respect to number of oocytes retrieved. This finding was observed not only for the overall trial population, but also for each of the two AMH strata used at randomisation.

A significant dose-response for FE 999049 was demonstrated for all key objective pharmacodynamic parameters, e.g. estradiol, inhibin B and inhibin A. At a similar microgram dose level, the pharmacodynamic responses with FE 999049 were larger than with GONAL-F (these results not shown).

The serum FSH concentrations after exposure to FE 999049 were significantly higher than for GONAL-F. The results confirm that the PK profile of FE 999049 differs from that of GONAL-F. Fertilisation rates, blastocyst develop-

ment and pregnancy rates in IVF/ICSI patients treated with FE 999049 were within expectations.

There were no safety concerns with the use of FE 999049. A good local tolerability was documented.

Further Analysis

The applicants have further analysed the data to identify the FE 999049 dose(s) that fulfil the following criteria with respect to number of oocytes retrieved:

Oocytes retrieved in the range 8-14

Minimise proportion of patients with <8 oocytes

Minimise proportion of patients with ≥20 oocytes

The applicants also investigated the impact of body weight. If relevant, the dose is converted into μg/kg for an average subject. This value of μg/kg and ±0.01 μg/kg are evaluated in a model with respect to distribution of oocytes retrieved as well as safety profile, and the optimal dose is identified.

Low AMH Strata

As seen in Table 2, the dose of FE999049 which fulfilled the first criterion (Oocytes retrieved in the range 8-14) was 12.1 μg (mean 9.4 oocytes retrieved). The distribution of oocytes is shown in Table 3 below.

TABLE 3

	FE 999049						GONAL-F
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg		11.3 (11) μg
Oocytes retrieved							
<4	32%	24%	15%	10%	10%		6%
4-7	63%	42%	45%	60%	20%		56%
8-14	5%	24%	35%	30%	60%	↔	33%
15-19	0%	5%	5%	0%	5%		6%
≥20	0%	5%	0%	0%	5%		0%

Data are % of subjects

As shown by the arrow, a dose of 12.1 μg FE999049 provides retrieval of the most desirable number of oocytes in 60% of subjects in the low AMH group. This is a marked improvement on Gonal-F (most desirable number of oocytes in only 33% of subjects).

Table 4 below shows the analysis of signs of excessive response in the low AMH strata (data are number of subjects). It can be seen that there were no indications of early OHSS of a moderate or severe nature and there were no incidences of preventative action being required; there are no concerns associated with the dose of 12.1 μg FE999049 in a patient having low AMH.

TABLE 4

	FE 999049					GONAL-F 11.3 (11)
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg	μg
All subjects	19	19	20	20	21	18
Early OHSS, mod/sev	0	0	0	0	0	0
GnRH agonist triggering	0	0	0	0	0	0
Preventive action*	0	0	0	0	0	0
≥15 oocytes	0	2	1	0	2	1
Any of the above	0	2	1	0	2	1

FIG. **7** shows the effect of body weight on oocytes retrieved (for the low AMH strata), for the various doses. The arrows indicate the number of oocytes retrieved from subjects of bodyweight between 45 kg and 90 kg treated at the 12.1 μg dose. As can be seen the variation between patients of bodyweight 45 kg and those of 90 kg is less than around 0.5 oocytes; in other words dosing by body weight is not required in patients with low AMH when dose of FE999049 is at least 12 μg, because there is not a significant variation in oocytes retrieved with body weight, at this dose.

Thus the applicants have found that a dose of, or dose equivalent to, 6 to 18 μg, for example 9 to 14 μg, for example 12 μg, human derived recombinant FSH is suitable for use in the treatment of infertility in a patient having serum AMH<15 pmol/L, for example 0.05-14.9 pmol/L for example 5.0-14.9 pmol/L. The dose provides an effective response while minimising risk of OHSS.

High AMH Strata

As seen in Table 2, three doses of FE999049 fulfilled the first criterion (oocytes retrieved in the range 8-14): 6.9 μg (mean 9.1 oocytes retrieved), 8.6 μg (mean 10.6 oocytes retrieved), and 10.3 μg (mean 13.6 oocytes retrieved).

FIG. **8** shows the effect of body weight on oocytes retrieved (for the high AMH strata), for the various doses. The arrows indicate the number of oocytes retrieved from subjects of body weight between 45 kg and 90 kg treated at the 6.9 μg, 8.6 μg and 10.3 μg doses. As can be seen the variation is significant: for the 6.9 μg dose 6 additional oocytes will be retrieved from a 45 kg patient compared to a 90 kg patient; for the 8.6 μg dose 4 additional oocytes will be retrieved from a 45 kg patient compared to a 90 kg patient; and for the 10.1 μg dose 2.5 additional oocytes will be retrieved from a 45 kg patient compared to a 90 kg patient. In other words dosing by body weight has an impact in patients with high AMH when the dose of FE999049 is less than 12 μg, because there is a significant variation in oocytes retrieved with body weight, at these doses.

Table 5a below shows a further breakdown of oocytes retrieved (from Table 2) by AMH. This shows the doses which fulfilled the first criterion (oocytes retrieved in the range 8-14) for each sub strata of AMH.

TABLE 5a

	FE 999049				
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg
Oocytes retrieved					
15-24 pmol/L	4.9 (3.8)	7.3 (3.6)	10.6 (5.1)	11.5 (6.7)	12.3 (5.9)
25-34 pmol/L	7.0 (1.8)	9.1 (6.8)	9.7 (6.7)	15.5 (6.4)	16.7 (4.9)
35-45 pmol/L	8.5 (9.2)	21.0 (1.4)	11.3 (2.6)	18.0 (12.2)	15.7 (6.5)

Table 5 b below shows the analysis of patients where treatment was cancelled due to either excessive response or agonist triggering, for these subgroups. For example, one patient in the 25-34 pmol/L AMH strata cancelled due to excessive response following the dose of 10.3 μg and one patient in the 25-34 pmol/L AMH strata cancelled due to excessive response following the dose of 12.1 μg; one patient in the 35-45 pmol/L AMH strata cancelled following agonist triggering following dose of 10.3 μg; and one patient in the 35-45 pmol/L AMH strata cancelled following agonist triggering following dose of 6.9 μg.

TABLE 5b

	FE 999049				
	5.2 μg	6.9 μg	8.6 μg	10.3 μg	12.1 μg
OHSS***, cancellation due to excessive response or agonist triggering****					
15-24 pmol/L	0	0	0	0	0
25-34 pmol/L	0	0	0	1***	1***
35-45 pmol/L	0	1****	0	1****	0

It can be seen therefore that tailoring of dose by bodyweight (FIG. **8**) and AMH level would be useful in the high AMH strata, to minimise cancellations and maximise oocyte retrieval.

The applicants have found that the following doses provide an effective response while minimising risk of OHSS (kg is kg body weight of patient).

Serum AMH	dose	(Max dose)
<15 pmol/L	12 μg	(12 μg)
15-24 pmol/L	0.14-0.19 μg/kg, for example 0.15-0.16 μg/kg, preferably 0.15 μg/kg	(12 μg)
25-34 pmol/L	0.11-0.14 μg/kg; for example 0.12-0.13 μg/kg, preferably 0.13 μg/kg	(12 μg)
≥35 pmol/L	0.10-0.11 μg/kg, preferably 0.11 μg/kg	(12 μg)

The following are appropriate if dosing by bodyweight is not desired.

Serum AMH	dose	(Max dose)
<15 pmol/L	12 μg	12 μg
15-24 pmol/L	9.3-10 μg	(12 μg)
25-34 pmol/L	7.3-8 μgμg	(12 μg)
≥35 pmol/L	6.3-7	(12 μg)

The following are appropriate if fewer categories of AMH are required.

4 AMH categories		3 AMH categories		2 AMH categories		One dose	
AMH	Dose	AMH	Dose	AMH	Dose	AMH	Dose
<15	12 μg	<15	12 μg	<15	12 μg	—	0.16 μg/kg
15-24	0.15-0.16 μg/kg	15-24	0.15-0.16 μg/kg	≥15	0.14 μg/kg		
25-34	0.12-0.13 μg/kg	≥25	0.12 μg/kg				
≥35	0.10-0.11 μg/kg						

The following are appropriate if dosing by bodyweight is not desired.

4 AMH categories		3 AMH categories		2 AMH categories		One dose	
AMH	Dose	AMH	Dose	AMH	Dose	AMH	Dose
<15	12 μg	<15	12 μg	<15	12 μg	—	9.3 μg or 10 μg
15-24	9.3-10 μg	15-24	9.3-10 μg	≥15	8.7 μg		
25-34	7.3-8 μg	≥25	7.3 μg				
≥35	6.3-7 μg						

Thus the applicants have found that a dose of, or dose equivalent to, 9 to 14 μg, for example 12 μg, human derived recombinant FSH is suitable for use in the treatment of infertility in a patient having serum AMH<15 pmol/L, for example 0.05-14.9 pmol/L for example 5.0-14.9 pmol/L. The dose provides an effective response while minimising risk of OHSS.

The applicants have found that a dose of, or dose equivalent to, 5 to 12.5 μg, for example 6 to 10.5 μg, human derived recombinant FSH is suitable for use in the treatment of infertility in a patient having serum AMH≥15 pmol/L. The dose provides an effective response while minimising risk of OHSS.

The applicants have found that a (e.g. daily) dose of, or dose equivalent to, 0.09 to 0.19 μg human derived recombinant FSH per kg bodyweight of the patient is suitable for use in the treatment of infertility in a patient having serum AMH level of ≥15 pmol/L. The applicants have found that a (e.g. daily) dose of, or dose equivalent to, 0.14 to 0.19 μg human derived recombinant FSH (preferably 0.15 to 0.16 μg human derived recombinant FSH) per kg bodyweight of the patient is suitable for use in the treatment of infertility in a patient having serum AMH level of 15 to 24.9 pmol/L. The applicants have found that a (e.g. daily) dose of, or dose equivalent to, 0.11 to 0.14 μg human derived recombinant FSH (preferably 0.12 to 0.13 μg human derived recombinant FSH) per kg bodyweight of the patient is suitable for use in the treatment of infertility in a patient having serum AMH level of 25 to 34.9 pmol/L. The applicants have found that a (e.g. daily) dose of, or dose equivalent to, 0.10 to 0.11 μg human derived recombinant FSH per kg bodyweight of the patient is suitable for use in the treatment of infertility in a patient having serum AMH level of 35 pmol/L. These doses provide an effective response while minimising risk of OHSS.

The applicants have found that a (e.g. daily) dose of, or dose equivalent to, 0.15 to 0.21 μg (e.g. 0.16 μg) human derived recombinant FSH per kg bodyweight of the patient is suitable for use in the treatment of infertility in a patient having serum AMH level of <15 pmol/L, for example for the first stimulation cycle with human derived recombinant FSH. However, it is not required that patients are dosed by body weight at this level of AMH.

Example 10 A—Individualised COS Protocol (Low AMH)

The selected patients are about to undergo COS for in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) by methods known in the art. The pre-treatment protocol includes assessment/screening of the patient's serum AMH using the AMH Gen-II enzyme linked immunosorbent assay kit (Beckman Coulter, Inc., Webster, Tex.). This assay can detect AMH concentrations greater than 0.57 pmol/L with a minimum limit of quantitation of 1.1 pmol/L. AMH may be measured using other Assay kits (e.g. available from Roche).

The COS protocol proceeds in the usual manner apart from administration of the initial dose of FE 999049 according to AMH level at screening. A patient with an AMH level of <14.9 pmol/L would be administered an initial daily dose of approximately 12 μg FE 999049, a human derived recombinant FSH product manufactured according to the method of Example 6. A patient with an AMH level of 15 to 24.9 pmol/L would receive an initial daily dose of 0.15 to 0.19 μg of the human derived recombinant FSH per kg bodyweight of the patient. A patient with an AMH level of 25 to 34.9 pmol/L would receive an initial daily dose of 0.11 to 0.13 μg of the human derived recombinant FSH per kg bodyweight of the patient. A patient with an AMH level of ≥35 pmol/L would receive an initial daily dose of 0.10 to 0.11 μg of the human derived recombinant FSH per kg bodyweight of the patient.

Example 11—Individualised COS Protocols

The doses in this protocol are less preferred that Example 10A.

The selected patients are about to undergo COS for in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) by methods known in the art. The pre-treatment protocol includes assessment/screening of the patient's serum AMH using the AMH Gen-II enzyme linked immunosorbent assay kit (Beckman Coulter, Inc., Webster, Tex.). This assay can detect AMH concentrations greater than 0.57 pmol/L with a minimum limit of quantitation of 1.1 pmol/L.

The COS protocol proceeds in the usual manner apart from administration of the initial dose of FE 999049 according to AMH level at screening in line with the following table. Thus a patient with an AMH level of 5-14.8 pmol/L would be administered 180 IU FSH in the form of approximately 8-11 μg FE 999049, a human derived recombinant FSH product manufactured according to the method of Example 6. A patient with an AMH level of 30-44.9 pmol/L would be administered 120 IU FSH in the form of approximately 4-7 μg FE 999049, a human derived recombinant FSH product manufactured according to the method of Example 6. If the AMH level is not available, the patient recombinant would be administered 120-180 IU FSH in the form of approximately 6-11 μg FE 999049, a human derived recombinant FSH product manufactured according to the method of Example 6.

AMH Level	Starting Dose FSH	Approx equivalent in μg
<5 pmol/l	210 IU	10-15 μg
5-14.9 pmol/l	180 IU	8-11 μg
>15-29.9 pmol/l	150 IU	6-9 μg
>30-44.9 pmol/l	120 IU	4-7 μg
>45 pmol/l	90 IU	2-5 μg
Not Available	120-180 IU	6-11 μg

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Follicle stimulating hormone alpha polypeptide
Accession number AH007338
Nucleotide sequence of FSH alpha

SEQ ID NO: 1

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  61 GTTCTCCATT CCGCTCCTGA TGTGCAGGAT TGCCCAGAAT GCACGCTACA GGAAAACCCA

 121 TTCTTCTCCC AGCCGGGTGC CCCAATACTT CAGTGCATGG GCTGCTGCTT CTCTAGAGCA

 181 TATCCCACTC CACTAAGGTC CAAGAAGACG ATGTTGGTCC AAAAGAACGT CACCTCAGAG

 241 TCCACTTGCT GTGTAGCTAA ATCATATAAC AGGGTCACAG TAATGGGGGG TTTCAAAGTG

 301 GAGAACCACA CGGCGTGCCA CTGCAGTACT TGTTATTATC ACAAATCTTA A
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Protein sequence of FSH alpha

(SEQ ID NO: 5)

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  61 YPTPLRSKKT MLVQKNVTSE STCCVAKSYN RVTVMGGFKV ENHTACHCST CYYHKS
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Follicle stimulating hormone beta polypeptide
Accession number NM _000510
Nucleotide sequence of FSH beta

SEQ ID NO: 2

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  61 TGTGAGCTGA CCAACATCAC CATTGCAATA GAGAAAGAAG AATGTCGTTT CTGCATAAGC

 121 ATCAACACCA CTTGGTGTGC TGGCTACTGC TACACCAGGG ATCTGGTGTA TAAGGACCCA

 181 GCCAGGCCCA AAATCCAGAA AACATGTACC TTCAAGGAAC TGGTATATGA AACAGTGAGA

 241 GTGCCCGGCT GTGCTCACCA TGCAGATTCC TTGTATACAT ACCCAGTGGC CACCCAGTGT

 301 CACTGTGGCA AGTGTGACAG CGACAGCACT GATTGTACTG TGCGAGGCCT GGGGCCCAGC

 361 TACTGCTCCT TTGGTGAAAT GAAAGAATAA
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Protein sequence of FSH beta

(SEQ ID NO: 6)

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   1 MKTLQFFFLF CCWKAICCNS CELTNITIAI EKEECRFCIS INTTWCAGYC YTRDLVYKDP

  61 ARPKIQKTCT FKELVYETVR VPGCAHHADS LYTYPVATQC HCGKCDSDST DCTVRGLGPS

 121 YCSFGEMKE
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Beta-galactoside alpha-2,3-sialyltransferase 4
Accession Number L23767
Nucleotide sequence ofST3GAL4

SEQ ID NO: 3

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 121 AAGAAGGAGC CGTGCCTCCA GGGTGAGGCA GAGAGCAAGG CCTCTAAGCT CTTTGGCAAC

 181 TACTCCCGGG ATCAGCCCAT CTTCCTGCGG CTTGAGGATT ATTTCTGGGT CAAGACGCCA

 241 TCTGCTTACG AGCTGCCCTA TGGGACCAAG GGGAGTGAGG ATCTGCTCCT CCGGGTGCTA

 301 GCCATCACCA GCTCCTCCAT CCCCAAGAAC ATCCAGAGCC TCAGGTGCCG CCGCTGTGTG

 361 GTCGTGGGGA ACGGGCACCG GCTGCGGAAC AGCTCACTGG GAGATGCCAT CAACAAGTAC

 421 GATGTGGTCA TCAGATTGAA CAATGCCCCA GTGGCTGGCT ATGAGGGTGA CGTGGGCTCC
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541 AACCCAGACA CACTCCTCGT CCTGGTAGCT TTCAAGGCAA TGGACTTCCA CTGGATTGAG

601 ACCATCCTGA GTGATAAGAA GCGGGTGCGA AAGGGTTTCT GGAAACAGCC TCCCCTCATC

661 TGGGATGTCA ATCCTAAACA GATTCGGATT CTCAACCCCT TCTTCATGGA GATTGCAGCT

721 GACAAACTGC TGAGCCTGCC AATGCAACAG CCACGGAAGA TTAAGCAGAA GCCCACCACG

781 GGCCTGTTGG CCATCACGCT GGCCCTCCAC CTCTGTGACT TGGTGCACAT TGCCGGCTTT

841 GGCTACCCAG ACGCCTACAA CAAGAAGCAG ACCATTCACT ACTATGAGCA GATCACGCTC

901 AAGTCCATGG CGGGGTCAGG CCATAATGTC TCCCAAGAGG CCCTGGCCAT TAAGCGGATG

961 CTGGAGATGG GAGCTATCAA GAACCTCACG TCCTTCTGA

Protein Sequence of ST3GAL4

(SEQ ID NO: 7)

1 MCPAGWKLLA MLALVLVVMV WYSISREDRY IELFYFPIPE KKEPCLQGEA ESKASKLFGN

61 YSRDQPIFLR LEDYFWVKTP SAYELPYGTK GSEDLLLRVL AITSSSIPKN IQSLRCRRCV

121 VVGNGHRLRN SSLGDAINKY DVVIRLNNAP VAGYEGDVGS KTTMRLFYPE SAHFDPKVEN

181 NPDTLLVLVA FKAMDFHWIE TILSDKKRVR KGFWKQPPLI WDVNPKQIRI LNPFFMEIAA

241 DKLLSLPMQQ PRKIKQKPTT GLLAITLALH LCDLVHIAGF GYPDAYNKKQ TIHYYEQITL

301 KSMAGSGHNV SQEALAIKRM LEMGAIKNLT SF

Beta-galactosamide alpha-2,6-sialyltransferase 1
Accession number NM _003032
Nucleotide sequence of ST6GAL1

SEQ ID NO: 4

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61 GCAGTCATCT GTGTGTGGAA GGAAAAGAAG AAAGGGAGTT ACTATGATTC CTTTAAATTG

121 CAAACCAAGG AATTCCAGGT GTTAAAGAGT CTGGGGAAAT TGGCCATGGG GTCTGATTCC

181 CAGTCTGTAT CCTCAAGCAG CACCCAGGAC CCCCACAGGG GCCGCCAGAC CCTCGGCAGT

241 CTCAGAGGCC TAGCCAAGGC CAAACCAGAG GCCTCCTTCC AGGTGTGGAA CAAGGACAGC

301 TCTTCCAAAA ACCTTATCCC TAGGCTGCAA AAGATCTGGA AGAATTACCT AAGCATGAAC

361 AAGTACAAAG TGTCCTACAA GGGGCCAGGA CCAGGCATCA AGTTCAGTGC AGAGGCCCTG

421 CGCTGCCACC TCCGGGACCA TGTGAATGTA TCCATGGTAG AGGTCACAGA TTTTCCCTTC

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541 TGGGGCAGGT GTGCTGTTGT GTCGTCAGCG GGATCTCTGA AGTCCTCCCA ACTAGGCAGA

601 GAAATCGATG ATCATGACGC AGTCCTGAGG TTTAATGGGG CACCCACAGC CAACTTCCAA

661 CAAGATGTGG GCACAAAAAC TACCATTCGC CTGATGAACT CTCAGTTGGT TACCACAGAG

721 AAGCGCTTCC TCAAAGACAG TTTGTACAAT GAAGGAATCC TAATTGTATG GGACCCATCT

781 GTATACCACT CAGATATCCC AAAGTGGTAC CAGAATCCGG ATTATAATTT CTTTAACAAC

841 TACAAGACTT ATCGTAAGCT GCACCCCAAT CAGCCCTTTT ACATCCTCAA GCCCCAGATG

901 CCTTGGGAGC TATGGGACAT TCTTCAAGAA ATCTCCCCAG AAGAGATTCA GCCAAACCCC

961 CCATCCTCTG GGATGCTTGG TATCATCATC ATGATGACGC TGTGTGACCA GGTGGATATT

1021 TATGAGTTCC TCCCATCCAA GCGCAAGACT GACGTGTGCT ACTACTACCA GAAGTTCTTC

1081 GATAGTGCCT GCACGATGGG TGCCTACCAC CCGCTGCTCT ATGAGAAGAA TTTGGTGAAG

1141 CATCTCAACC AGGGCACAGA TGAGGACATC TACCTGCTTG GAAAAGCCAC ACTGCCTGGC

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Op-Protein Sequence of ST6GAL1

(SEQ ID NO: 8)

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  61 QSVSSSSTQD PHRGRQTLGS LRGLAKAKPE ASFQVWNKDS SSKNLIPRLQ KIWKNYLSMN

 121 KYKVSYKGPG PGIKFSAEAL RCHLRDHVNV SMVEVTDFPF NTSEWEGYLP KESIRTKAGP

 181 WGRCAVVSSA GSLKSSQLGR EIDDHDAVLR FNGAPTANFQ QDVGTKTTIR LMNSQLVTTE

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 301 PWELWDILQE ISPEEIQPNP PSSGMLGIII MMTLCDQVDI YEFLPSKRKT DVCYYYQKFF

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SEQUENCE LISTING

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gccaggccca aaatccagaa aacatgtacc ttcaaggaac tggtatatga aacagtgaga    240

gtgcccggct gtgctcacca tgcagattcc ttgtatacat acccagtggc cacccagtgt    300

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35 40 45

Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro
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Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu
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Glu Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr Trp Cys Ala Gly
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Tyr Cys Tyr Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala Arg Pro Lys
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Ile Gln Lys Thr Cys Thr Phe Lys Glu Leu Val Tyr Glu Thr Val Arg
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Val Pro Gly Cys Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro Val
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Leu Phe Tyr Phe Pro Ile Pro Glu Lys Lys Glu Pro Cys Leu Gln Gly
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Gln Pro Ile Phe Leu Arg Leu Glu Asp Tyr Phe Trp Val Lys Thr Pro
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Ser Ala Tyr Glu Leu Pro Tyr Gly Thr Lys Gly Ser Glu Asp Leu Leu
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Leu Arg Val Leu Ala Ile Thr Ser Ser Ser Ile Pro Lys Asn Ile Gln
            100                 105                 110

Ser Leu Arg Cys Arg Arg Cys Val Val Val Gly Asn Gly His Arg Leu
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Arg Asn Ser Ser Leu Gly Asp Ala Ile Asn Lys Tyr Asp Val Val Ile
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Arg Leu Asn Asn Ala Pro Val Ala Gly Tyr Glu Gly Asp Val Gly Ser
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Lys Thr Thr Met Arg Leu Phe Tyr Pro Glu Ser Ala His Phe Asp Pro
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Lys Val Glu Asn Asn Pro Asp Thr Leu Leu Val Leu Val Ala Phe Lys
            180                 185                 190

Ala Met Asp Phe His Trp Ile Glu Thr Ile Leu Ser Asp Lys Lys Arg
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Val Arg Lys Gly Phe Trp Lys Gln Pro Pro Leu Ile Trp Asp Val Asn
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Pro Lys Gln Ile Arg Ile Leu Asn Pro Phe Phe Met Glu Ile Ala Ala
225                 230                 235                 240

Asp Lys Leu Leu Ser Leu Pro Met Gln Gln Pro Arg Lys Ile Lys Gln
                245                 250                 255

Lys Pro Thr Thr Gly Leu Leu Ala Ile Thr Leu Ala Leu His Leu Cys
            260                 265                 270

Asp Leu Val His Ile Ala Gly Phe Gly Tyr Pro Asp Ala Tyr Asn Lys
        275                 280                 285

Lys Gln Thr Ile His Tyr Tyr Glu Gln Ile Thr Leu Lys Ser Met Ala
    290                 295                 300

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            20                  25                  30

Ser Tyr Tyr Asp Ser Phe Lys Leu Gln Thr Lys Glu Phe Gln Val Leu
        35                  40                  45

Lys Ser Leu Gly Lys Leu Ala Met Gly Ser Asp Ser Gln Ser Val Ser
    50                  55                  60

Ser Ser Ser Thr Gln Asp Pro His Arg Gly Arg Gln Thr Leu Gly Ser
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Leu Arg Gly Leu Ala Lys Ala Lys Pro Glu Ala Ser Phe Gln Val Trp
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               85                  90                  95

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Trp Lys Asn Tyr Leu Ser Met Asn Lys Tyr Lys Val Ser Tyr Lys Gly
        115                 120                 125

Pro Gly Pro Gly Ile Lys Phe Ser Ala Glu Ala Leu Arg Cys His Leu
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Arg Asp His Val Asn Val Ser Met Val Glu Val Thr Asp Phe Pro Phe
145                 150                 155                 160

Asn Thr Ser Glu Trp Glu Gly Tyr Leu Pro Lys Glu Ser Ile Arg Thr
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Lys Ala Gly Pro Trp Gly Arg Cys Ala Val Val Ser Ser Ala Gly Ser
            180                 185                 190

Leu Lys Ser Ser Gln Leu Gly Arg Glu Ile Asp Asp His Asp Ala Val
        195                 200                 205

Leu Arg Phe Asn Gly Ala Pro Thr Ala Asn Phe Gln Gln Asp Val Gly
    210                 215                 220

Thr Lys Thr Thr Ile Arg Leu Met Asn Ser Gln Leu Val Thr Thr Glu
225                 230                 235                 240

Lys Arg Phe Leu Lys Asp Ser Leu Tyr Asn Glu Gly Ile Leu Ile Val
                245                 250                 255

Trp Asp Pro Ser Val Tyr His Ser Asp Ile Pro Lys Trp Tyr Gln Asn
            260                 265                 270

Pro Asp Tyr Asn Phe Phe Asn Asn Tyr Lys Thr Tyr Arg Lys Leu His
        275                 280                 285

Pro Asn Gln Pro Phe Tyr Ile Leu Lys Pro Gln Met Pro Trp Glu Leu
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Trp Asp Ile Leu Gln Glu Ile Ser Pro Glu Glu Ile Gln Pro Asn Pro
305                 310                 315                 320

Pro Ser Ser Gly Met Leu Gly Ile Ile Ile Met Met Thr Leu Cys Asp
                325                 330                 335

Gln Val Asp Ile Tyr Glu Phe Leu Pro Ser Lys Arg Lys Thr Asp Val
            340                 345                 350

Cys Tyr Tyr Tyr Gln Lys Phe Phe Asp Ser Ala Cys Thr Met Gly Ala
        355                 360                 365

Tyr His Pro Leu Leu Tyr Glu Lys Asn Leu Val Lys His Leu Asn Gln
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Gly Thr Asp Glu Asp Ile Tyr Leu Leu Gly Lys Ala Thr Leu Pro Gly
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The invention claimed is:

1. A method of treating infertility, comprising administering recombinant follicle stimulating hormone (FSH) that includes α-2,3- and α 2,6-sialylation to a patient in need of such treatment, wherein the FSH is administered at a daily dose of, or a dose equivalent to a daily dose of, 11 to 13 μg of said recombinant FSH, wherein the patient has a serum anti-mullerian (AMH) hormone level of <15 pmol/L, and wherein the FSH is administered together with a gonadotropin releasing hormone (GnRH) antagonist.

2. The method of claim 1, wherein the method comprises a step of determining the serum AMH level of the patient, and a step of administering the dose of FSH to the patient having the serum AMH level of <15 pmol/L.

3. A method of treating infertility, comprising administering recombinant follicle stimulating hormone (FSH) that includes α-2,3- and α 2,6-sialylation to a patient in need of such treatment, wherein the FSH is administered at a daily dose of, or a daily dose equivalent to a daily dose of, 0.09 to 0.19 μg of said recombinant FSH per kilogram body weight of the patient, wherein the patient has a serum anti-mullerian hormone level of ≥15 pmol/L, and wherein the FSH is administered together with a gonadotropin releasing hormone (GnRH) antagonist.

4. The method of claim 3, wherein the method comprises a step of determining the serum AMH level of the patient, and a step of administering the dose of FSH to the patient having the serum AMH level of ≥15 pmol/L.

5. A method of treating infertility, comprising administering recombinant follicle stimulating hormone (FSH) that includes α-2,3- and α 2,6-sialylation to a patient in need of such treatment, wherein the FSH is administered at a daily dose of, or a dose equivalent to a daily dose of, 11 to 13 μg of said recombinant FSH, wherein the patient has a serum anti-mullerian (AMH) hormone level of <15 pmol/L, and wherein the FSH is administered together with human chorionic gonadotropin (hCG).

6. The method of claim 5, wherein the method comprises a step of determining the serum AMH level of the patient, and a step of administering the dose of FSH to the patient having the serum AMH level of <15 pmol/L.

7. A method of treating infertility, comprising administering recombinant follicle stimulating hormone (FSH) that includes α-2,3- and α 2,6-sialylation to a patient in need of such treatment, wherein the FSH is administered at a daily dose of, or a daily dose equivalent to a daily dose of, 0.09 to 0.19 μg of said recombinant FSH per kilogram body weight of the patient, wherein the patient has a serum anti-mullerian hormone (AMH) level of ≥15 pmol/L, and wherein the FSH is administered together with human chorionic gonadotropin (hCG).

8. The method of claim 7, wherein the method comprises a step of determining the serum AMH level of the patient, and a step of administering the dose of FSH to the patient having the serum AMH level of ≥15 pmol/L.

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